

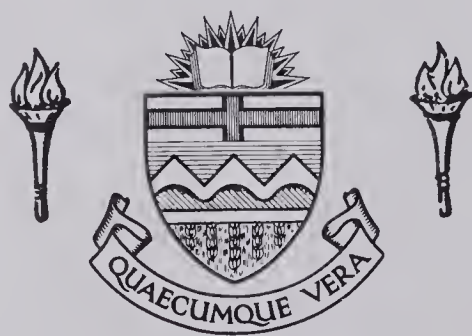
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THE UNIVERSITY OF ALBERTA

MORPHOLOGY AND INNERVATION OF MUSCLE
SPINDLES IN THE LUMBRICAL MUSCLES OF THE RAT

A thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements for

the Degree of
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Faculty of Medicine

Department of Surgery

By



Orest Porayko B.Sc., M.D.

Edmonton, Alberta

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UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Morphology and Innervation of Muscle Spindles in the Lumbrical Muscles of the Rat", submitted by Orest Porayko in partial fulfillment of the requirements for the degree of Master of Science (Surgery).

ABSTRACT

The study of mammalian muscle spindles has been in progress for about 100 years. Up to the present, considerable controversy exists as to the motor innervation of the individual intrafusal muscle fibers. Since this problem has not been resolved in mammals studied thus far, then it is clear that other species must be examined.

The morphology and the innervation of muscle spindles found in the plantar lumbrical muscles of the rat's hindpaw were studied. Non-nervous aspects of muscle spindle morphology were examined by reconstruction of 14 spindles from serial cross sections of lumbrical muscles. The structure of the spindles was similar to that found in other mammals. Each spindle contained three to five intrafusal fibers. Most of the spindles contained two nuclear bag and two nuclear chain fibers. These muscle fibers could be distinguished by their dimensions. There was, however, some overlap of diameter and considerable overlap of length as the nuclear chain fibers consistently tended to extend beyond the spindle capsule poles. No branching of the intrafusal fibers was found. Equatorial nuclear aggregations, and variations in the nuclear shape, allowed for identification of muscle fiber type.

The receptor content of each of the six lumbrical muscles was determined. Through degeneration experiments, the nerve fiber content in each of the lumbrical nerve trunks was ascertained. The motor fibers were consistently smaller than the sensory fibers and made up about one third of the total nerve fiber content in each muscle

nerve. There was a ratio of about one motor axon, in the muscle nerve, for each spindle found in the muscle. Stimulation of the muscle nerve revealed the presence of three to five extrafusal motor units. Conduction velocities recorded from the muscle nerve of lumbrical 5 confirmed the nerve fiber spectra obtained histologically.

The innervation of muscle spindles was examined in silver stained, teased spindles. Two motor axons, which differed in diameter, entered each spindle and innervated nuclear bag and nuclear chain fibers through two different types of motor endings. Though no consistent specificity was present, the thicker motor axon frequently innervated the nuclear bag fiber with plate endings and the thinner motor axon innervated the nuclear chain fiber with filamentous endings. The motor innervation to the two types of intrafusal fiber remained separate in all specimens which were examined. Skeletofusimotor, or beta innervation, and branching of fusimotor axons, to innervate intrafusal fibers in more than one spindle, were present. Although their frequency of occurrence was not ascertained, they served to explain how each of four to eight spindles could receive two motor axons when only nine motor fibers were present in the muscle nerve trunk and with at least three to five extrafusal motor units present.

Sensory nerve endings in the spindles, and their nerves, were similar to those described in other mammals. The afferent response to stretch of the muscle had a characteristic dynamic and static phase. Increased afferent discharge, with addition of succinylcholine, was also recorded. Limited extrafusal fiber activation, with addition of succinylcholine, was noted.

From information revealed in this study, and that extrapolated from the literature, one may conclude that the nuclear bag and nuclear chain fibers are different morphologically and functionally and therefore act independently of each other.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES AND FIGURES	vii
CHAPTER I INTRODUCTION	1
1. Histological Review	6
2. Physiological Review	15
3. Summary of Histological Review	23
and Aim of Project	
CHAPTER I I METHODS AND MATERIALS	26
1. Operative Technique	26
2. Histological Techniques	29
3. Physiological Techniques	36
CHAPTER III RESULTS	39
1. Gross Anatomy of the Nerve Supply to the Hindfoot	39
2. Morphology of Muscle Spindles	41
3. Composition of the Nerve Trunk to the Lumbrical Muscles	46
4. The Motor Innervation of Muscle Spindles	50
5. The Sensory Innervation	54
6. Physiological Characteristics of the Muscle Spindle	56

	Page
CHAPTER IV DISCUSSION AND SUMMARY.....	57
1. Structure of Muscle Spindles	57
2. Technical Problems Encountered	59
3. The Motor Innervation	60
4. The Motor Nerve Endings	62
5. The Motor Nerves	64
6. The Sensory Innervation	65
7. Other Nerve Fibers in the Spindle	66
8. Evidence for Two Morphological	67
Intrafusar Muscle Fiber Types	
SUMMARY	70
BIBLIOGRAPHY	73
TABLES AND FIGURES	81

LIST OF TABLES AND FIGURES

		Page
Table	1. Muscle weights, spindle content and muscle nerve composition of six lumbrical muscles	81
Figure	1. Sizes of plantar lumbrical muscles, their spindle receptor content, and their manner of origin	82
	2. Gross innervation of the hindpaw plantar lumbrical muscles	83
	3. Spindles reconstructed from serial cross sections of lumbrical muscles	84
	4. Cross sections of muscle spindles	85
	5. Low and high power photomicrographs of muscle cross sections	86
	6. Extrafusal muscle fiber diameter histograms obtained from lumbrical muscles fixed and stained by two different methods for calculation of shrinkage	87
	7. Extrafusal and intrafusal muscle fiber diameter distributions of muscles embedded in paraffin and stained with hematoxylin and eosin	88
	8. Muscle spindle length distribution as determined by two staining methods	89
	9. Intrafusal muscle fiber length histogram	90
	10. Comparison of nuclear dimensions taken from nuclear bag and nuclear chain fibers of the rat and cat	91
	11. Whole mounts of myelinated nerve fibers, stained with osmic acid, at various stages of degeneration	92
	12. Spindles teased, for control purposes, from muscles attached to the nerve trunks which were used to obtain nerve cross sections	93
	13. Cross sections of the nerve trunk to lumbrical muscle 5 with the control sections of the skin nerves to the 5th. digit	94

Figure		Page
14.	Cross sections of myelinated motor nerve fibers	95
15.	(a) (b). Nerve fiber diameter distributions of muscle nerves innervating the six plantar lumbrical muscles	96
16.	Histogram showing distribution of a random sample of nerve fibers contained in lumbar ventral roots 4,5,6	97
17.	Schematic representation of the apparatus in which stimulating and recording procedures were carried out	98
18.	Conduction characteristics of nerve fibers contained in the nerve to lumbrical muscle 5	99
19.	Contractile responses of lumbrical muscle 5	100
20.	Three examples of beta or skeletofusimotor innervation	101
21.	Fusimotor axon branching to innervate adjacent spindles	102
22.	Intercostal muscle spindles of the cat to show the quality of axon staining	103
23.	Examples of extra and intrafusal motor endings	104
24.	Fusimotor innervation at the polar ends of several spindles	105
25.	The entire motor innervation to one pole of a spindle	106
26.	(a)(b). Schematic representations of the motor innervation of four spindles	107
27.	Gross appearance and innervation of spindles at their equatorial region	108
28.	Primary sensory endings on nuclear bag and nuclear chain muscle fibers	109
29.	Primary endings on two simple spindles innervated by a common sensory axon	110
30.	Muscle spindles showing sensory, motor and autonomic nerve fibers, as well as blood vessels, in the capsular region	111

	Page
Figure 31. Discharge characteristics of spindle afferents contained in plantar lumbrical muscle 5	112

CHAPTER 1

INTRODUCTION

Introduction

Since the literature is so extensive and implications so wide, a general statement of the present thinking on the role that spindles play in the control of skeletal muscle and implications of this thinking will be presented here.

A more detailed and more specific survey of the literature will be presented in the sections dealing with the histological and physiological background.

It has long been suspected that the receptors of a muscle are vitally important for the integration by the nervous system necessary to bring about coordinated movement. Suspicions to this effect stemmed from the observations that the presence of sensory nerve endings in striated muscle were found to be widespread throughout the animal kingdom, though the endings were easier to identify in some species than in others. The most widely studied of these endings is the muscle spindle, whose history goes back over 100 years.

Little groups of small diameter muscle fibers were seen in vertebrate muscles about 1850; Kölliker (1862), using the frog, and Kühne (1863), using the mammal, showed that these groups were supplied by at least one large nerve fiber, often larger than the fibers to the motor end-plates. These groups of muscle fibers formed small entities, being partly encapsulated, and were called muscle spindles. Some people thought them to be pathological or developing muscle fibers, but their widespread occurrence suggested that they were normal structures. Sherrington frequently wrote about their sensory function and was convinced that they played an important part in muscle tone and in myotatic reflexes.

In the past, muscle spindles have been talked about in terms of an oversimplified model containing a single type of receptor and controlled by a single type of motor fiber. The modern concept is of a duality of the effector and receptor motor systems in the spindle. The evidence for this is so recent that full agreement has not yet been reached either on the histological details or on the physiological differences between the systems.

The muscle spindle may be considered as a device signaling mechanical events by means of two different outputs (the afferent fibers) and controlled by two additional inputs (the efferent fibers). As stretch receptors, they are unique in that they can be excited by their own motor fibers. Precise functions are far from being understood in view of their histological and physiological complexity.

In order that physiological data may be accurately interpreted, a clear, precise histological picture of the system under study must be present. The complex arrangement of the numerous nerve endings in muscle spindles has been studied by light microscopy for over 100 years, but agreement still has not been reached about the finer details of their structure. Electron microscopic study of muscle spindles is just starting (69,77).

Several investigatory methods have been adopted and are considered to be necessary for elucidation of muscle spindle morphology and innervation. Spindle reconstruction from serial cross sections through the muscle under study yields the most accurate picture on the length and diameter of intrafusal fibers, nuclear arrangement at the equator and the presence or absence of branching of the intrafusal muscle fibers. This is, however, a difficult and tedious procedure since most spindles

are several millimeters long. Degeneration experiments are necessary to separate motor, sensory and sympathetic fibers in the muscle nerve.

Teased whole mounts of muscle spindles have been studied extensively. Through this technique motor and sensory innervation of the intrafusal muscle fibers can be determined. Difficulties arise in areas where motor and sensory endings overlap, therefore degeneration studies are necessary for clarification. An important difficulty encountered in studying teased whole mounts of muscle spindles is the inconsistency of the classical gold and silver staining techniques necessary to show details of the fine axons and nerve endings within the spindle.

In the course of elucidation of muscle spindle morphology, muscles from several different creatures in the animal kingdom have been studied. Stretch receptors have been found in fish but were not typical of those found in mammals. The frog and toad have had their muscle spindles subjected to many histological and physiological investigations. Muscle spindles of snakes, lizards and tortoises were found to have a capsule and sensory and motor endings but varied from being unifascicular to multifascicular. Sensory endings in bird muscles resemble much more closely those in mammalian muscles in that they are encapsulated, multifascicular and richly supplied with nerve fibers.

Spindles have been found in all the numerous somatic limb and trunk muscles in which they have been sought, and this has been in a wide range of mammalian species (28,49). In certain muscles innervated by the cranial nerves however, no muscle spindles have been found even though they have been searched for carefully.

The best documented examples of muscles without muscle spindles are the extrinsic ocular muscles of many species (for example: cat, dog, rabbit, horse) although in other species (man, apes, goat, sheep) these muscles may contain many typical muscle spindles (29,78).

Cooper (28) has compiled, from the works of Voss, Schulze and Freimann, a chart showing the number of spindles in various limb and cranial muscles of man. She also indicates the distribution of spindles in non-joint moving muscles such as the striated esophageal muscles, cremaster muscle and the diaphragm.

The ultimate purpose in the intensive investigations related to muscle spindle structure and function is to determine what role it plays in normal movement and what part it plays in various neuromuscular disorders. The possible role of the efferent nerve fibers to the spindle in the production of certain symptoms in man has recently been discussed by several authors (50, 58, 68, 88, 93).

Histopathologically, spindles persist apparently unchanged in many muscular diseases. Long after some of the extrafusal muscle fibers have ceased to function and are in a state of degeneration, the intrafusal fibers may appear intact, and to the uninitiated, more numerous because of the loss of extrafusal muscle fibers. Most spindles show few, if any, changes in human cases of motor neuron disease and dorsal root ganglion disease probably because these diseases progress slowly and show patchy changes in various muscles.

Muscles do not change histologically with upper motor neuron disease. There are, however, disturbances of posture as a result of malfunction in the central pathways playing on the gamma motoneurons which supply the intrafusal muscle fibers of the muscle spindle.

More specifically, the pathophysiology of Parkinsonian rigidity has been attributed by Rushworth (88, 89) to an overactivity of the efferent nerve fibers to the spindle which produces oversensitization of muscle spindles to stretch. Jansen, from evidence obtained from decerebration experiments (59, 60, 61, 62), concludes that spastic states are due mainly to a selective activation of one functional component of the spindle over the other. With the evidence cited by the above authors, even in the absence of precise knowledge of the mechanisms of the effects produced, selective paralysis or destruction of efferent nerve fibers to the spindle may prove to be of value in the treatment of diseases accompanied by excessive muscular tone.

Histological Review

Several excellent reviews (28, 49, 52, 76, 97), trace the development of the mammalian muscle spindle over 100 years from the first description of Hassall in 1851 to Boyd's concept, in 1962, of duality of muscle fiber type and motor and sensory innervation.

I. Classical Picture of Spindle Structure

The early workers, including Kölliker and Kühne, thought that the spindle-shaped areas seen in skeletal muscle were growth centers and called them muscle buds. The concept that these spindle-shaped areas were centers of muscle regeneration was based on little more than the small size of the intrafusal muscle fibers and their large number of nuclei.

It remained for the experiments of Sherrington in 1894 (91), supported by the fully illustrated observations of Ruffini working during the period 1893 - 1898 (86, 87), to lead to general acceptance of the idea that muscle spindles were elaborately organized sense organs. Because the observations and conclusions of Ruffini and Sherrington form the background for the present day concept of muscle spindle morphology, a summary of their results will be presented.

Sherrington's concept was that of a spindle several millimeters long containing 2-12 intrafusal muscle fibers with diameters varying from 6-28 μ . The diameter of the spindle equator ranged from 80 - 200 μ . He thought that the periaxial capsular space contained lymph, as tracer dyes injected into lymphatics appeared here, however this has been recently doubted by Brezezinski (76). Intrafusal muscle fibers were noted to be striated at their polar ends with the

striations poorly shown in the central regions where nuclear aggregations were found.

Ruffini demonstrated 3 kinds of nerve ending. The primary or annulospiral ending was supplied by a single large nerve fiber which showed extensive pre-terminal branching before terminating as spirals wrapped around the individual intrafusal muscle fibers. The secondary or flowerspray ending lay beyond the primary ending and was supplied by a smaller nerve fiber. Its terminations were less regular than those of the primary and consisted of varicose filaments of diverse form running along the intrafusal muscle fibers. Discrete plate endings of varying size were the third type of nerve ending encountered. These endings were supplied by smaller nerve fibers than either the primary or secondary endings and were found in the polar region of the spindle.

It was noted that different muscle spindles differed in their complexity of innervation. Complex spindles had a single primary ending, end-plates at the spindle poles and two secondary endings both of which might lie at one end, or on either side, of the primary ending. The intermediate spindle had one each of primary and secondary endings also plate endings at the poles. The simple variety contained a single primary ending, several plate endings but no secondary endings.

The terms primary and secondary ending have come to be preferred to Ruffini's alternative terms (annulospiral and flower-spray ending) as the difference in appearance of the terminations is often not as marked as he described it; but the two types of ending may be differentiated by whether they lie on the central region of the intrafusal muscle fibers (primary), or nearer their poles (secondary) (2, 18).

Ruffini felt that his three types of endings were sensory. Sherrington (91), through his degeneration experiments, proved the sensory nature of the primary ending. He too was inclined to doubt whether the spindle had a motor supply at all. These two investigators apparently overlooked the work of Onanoff (76), who in 1890, did selective destruction of ventral roots and dorsal root ganglia in the dog and concluded that the spindle was a sense organ under motor control.

Degeneration experiments performed in the late 1920's by Hinsey (48) and Hines and Tower (47) showed clearly that the plate endings at the poles of the spindle were motor and that the central primary ending was sensory in nature. Hinsey (48) noted that the secondary ending persisted after ventral root section but degenerated after dorsal root ganglionectomy. Thus the classical picture of the spindle as it appeared by 1930 established it as a sense organ with two types of afferent ending, the primary and secondary, and one type of motor ending, the polar plates, and no sympathetic nerve supply.

The classical picture of the muscle spindle was fortified in 1948 when Barker (2) published a detailed study of normal muscle spindles, mainly those of the rabbit, which confirmed previous findings. Barker observed that the secondary ending had a less central position than the primary and was supplied by a smaller nerve fiber (6 - 9 μ rather than 8 - 12 μ diameter). He concluded that these criteria best differentiated primary and secondary endings rather than specific differences in the form of their terminations. He introduced the term nuclear bag for the dense

aggregation of nuclei in the central region of the intrafusal muscle fibers and the term myotube region for the parts of the intrafusal muscle fiber on either side of the nuclear bag where nuclei were concentrated in a single axial chain. He was able to show that the primary ending lies on the nuclear bag and the secondary ending on the myotube region of the intrafusal muscle fibers. Each intrafusal muscle fiber was noted to have a plate ending at either end.

Until very recently the above classical description of muscle spindles and their innervation had been accepted by most workers and was used in the interpretation of virtually all experimental physiological work.

2. Current Concepts of the Muscle Spindle

Within the last 10 - 15 years extensive investigations of the morphology and innervation of the mammalian muscle spindle has produced results which show that the classical picture just presented is too simple. The most notable contribution is the possibility that the motor supply to the spindle is double as well as the sensory supply. The original histochemical work of Cöers and Durand (26) was most suggestive in this respect. They stained cat spindles for choline esterase and found that except for a central region of 360 - 660 μ the whole length of the intrafusal bundle was stained. This suggested that the spindle had a motor innervation along most of its length, whereas the plate endings occur almost entirely in its polar regions. The staining was particularly dense in the juxta - equatorial regions of the spindle where the secondary endings would be expected to lie.

Recent work has revealed that intrafusal muscle fibers in man (30), in the opossum (63) and in the cat (3, 7, 17, 18, 23, 96) fall into two classes which are distinguished by differences in length, diameter, and in the arrangements of their nuclear aggregations at the equator of the muscle spindle. The longer, wider fibers are packed with nuclei at the equator and extend for a considerable distance beyond the poles of the spindle capsule; the shorter, narrower fibers have single chains of nuclei at the equator and do not extend much beyond the poles. Boyd (18) termed these two types "nuclear bag fibers" and "nuclear chain fibers" respectively. This duality seems to be characteristic of intrafusal fibers in all mammals except the rabbit, which has only nuclear bag fibers (8).

Barker and Gidumal (7) doubted that intrafusal fibers fell cleanly into the two classes mentioned above and described fibers of "intermediate" diameter containing small equatorial nuclear bags; such fibers were present in about one third of cat spindles. In observing fibers of a similar nature, also in the cat, Bridgman, Eldred and Eldred (23) felt that this did not rule against the general separation into two types. It is however possible that some intrafusal muscle fibers are not certainly classifiable as either a bag or a chain fiber, as would be hardly surprising in a biological system, especially since all the fibers are developed from common stem muscle fibers with central nuclei (31, 32).

Boyd (17) felt that the primary and secondary sensory endings could be distinguished from each other on morphological grounds. Large afferent fibers forming the primary sensory ending terminated as spirals around the central parts of the

nuclear bag and nuclear chain fibers. Secondary sensory endings were found as spirals and sprays mainly on the nuclear chain fibers but branches ending only as sprays on the myotube portion of the nuclear bag fiber were also found. This concept formed the basis for widespread agreement by most workers for morphological and physiological independence of primary and secondary endings.

A great deal of controversy has arisen following Boyd's (17, 18) suggestion that the two types of mammalian intrafusal muscle fiber have an entirely separate motor innervation. In the cat, for example, the nuclear bag and nuclear chain type intrafusal fibers were described by Boyd (17, 18) as receiving different and specific motor nerve endings. He found that typical motor end-plates occurred only on nuclear bag fibers (supplied by gamma-1 nerve fibers), usually near the poles of the spindle, and that diffuse networks of fine fibers occurred only on nuclear chain fibers (supplied by gamma-2 nerve fibers), usually near the equator. Boyd's observations seemed to confirm those of Hess (46) who, in the spindles of mice and rabbits, found cholinesterase distributed both as polar end-plates and as juxta-equatorial "diffuse multiterminal endings". Hess was unable to state, however, if the two types of ending lay on the same or different kinds of intrafusal fiber.

Barker (3, 5) disagreed with Boyd's thesis and maintained that both nuclear bag and nuclear chain fibers in the cat received motor end-plates only. The recent observation of diffuse trailing motor endings near the equator of cat and rabbit spindles by Barker and Ip (10) does not entirely resolve the controversy, for

they see in these "trail endings" some resemblances to configurations previously described by them (11) as "plate ending fibers carrying out end plate replacement". Barker feels that the branches of a single gamma fiber commonly supply both kinds of intrafusal fiber and concluded that "neither group of fibers is specific with respect to the type of muscle fiber it innervates" (5).

Barker and his colleagues (1, 3, 5, 10) have also denied Boyd's (17, 18) claim that nuclear bag end plates and nuclear chain networks are supplied by gamma efferent nerve fibers which are distinguished by specific differences in axon diameter.

In view of these direct contradictions, particularly with regard to the motor innervation, Boyd's picture of the cat spindle cannot be accepted as established. The main point at issue is whether or not different kinds of intrafusal muscle fibers are independently innervated. Therefore, detailed studies of spindle innervation in other species are clearly desirable to further the understanding of mammalian muscle spindle innervation as a whole.

3. Origin of Motor Fibers

In 1930 Eccles and Sherrington (34) showed a bimodal distribution of the diameters of the motor nerve fibers in the nerves of deafferented muscles in the cat. As a similar bimodal distribution of fiber sizes was also found for the motor fibers in the ventral roots, Eccles and Sherrington concluded that the smaller fibers (modal diameter $6\ \mu$) were largely a specific group and were not produced solely by division of the large fibers (modal diameter $15\ \mu$). Some small fibers in the muscle nerve did arise through branching of large fibers, which was found to occur fairly commonly, particularly as the nerve approached the muscle. They suggested that the

group of small fibers merely supplied smaller motor units than did the large fibers, though in 1922 Langley (70) had suggested that the small myelinated fibers in the ventral root might supply the muscle spindles. Physiological experiments have since supported this latter suggestion.

The fibers of origin of the gamma-1 and gamma-2 fibers of Boyd (18) have still not been established anatomically, and it is conceivable that one of these sets of fibers could be derived from alpha fibers; Boyd (18) produces various arguments against such a possibility. One anatomical finding bearing on this problem is that the gamma-2 fibers branch more than the gamma-1 fibers, so that even though they are of different size at the spindle they are about the same size in the intramuscular nerve bundles (into which their individual fibers can be traced), and "at this point it is difficult, and often impossible, to distinguish", gamma-1 and gamma-2 fibers in preparations stained with gold chloride (18). Boyd and Davey (20), however, have separated gamma fibers into two groups on the basis of the thickness of their myelin sheath stained with osmium tetroxide. These two groups were particularly well separated in the nerve to the popliteus muscle, where the well-myelinated fibers were appreciably thicker than the thinly myelinated fibers, giving a bimodal distribution of the sizes of the gamma fibers. Boyd and Davey (20) suggest that these two groups were "perhaps the stem fibers of the gamma-1 and gamma-2 motor fibers found at the spindles" but had no further observations in support of this idea.

Adal and Barker (1), on the other hand, suggest that the nature of the intramuscular branching of gamma fibers demonstrates that the gamma fibers do not

supply gamma-1 and gamma-2 fibers to the spindles in accordance with their stem diameters and degree of myelination. Since, apparently, there is no correlation between the size of a motor fiber or fiber branch entering a spindle and the type of intrafusal muscle fiber it innervates, as Barker and Cope (5) and Barker, Cope and Ip (6) maintain, then one must conclude that the diameters of gamma fibers, stem or branch, are of no particular significance; and that the simple duality of Boyd's classification of motor fibers entering the spindle into gamma-1 and gamma-2 fibers is not justified.

Partly because it occurs in some lower animals the suggestion has frequently been made that some of the motor fibers to the spindle are branches of the ordinary alpha motor fibers to the extrafusal muscle fibers. These motor fibers are called beta or skeletofusimotor fibers. Adal and Barker (1) have obtained histological evidence for this type of innervation by tracing the intramuscular course of 27 stem motor fibers. Five of these were found to branch to innervate both intra and extrafusal muscle fibers. Such branching, on histological grounds, has been considered uncommon, however recent physiological work by Granit, Pompeiano and Waltman (42, 43), Bessou, Emonet - Denand and Laporte (12, 13) and Brown, Crowe and Matthews (24) in the cat, and Steg (94) and Kidd (65) in the rat tail muscles suggests that this form of innervation may be more frequently observed were it not for the problems encountered in staining and teasing out the spindles with their innervation intact.

Physiological Review

Much of the work carried out by electrophysiological methods has, of necessity, been as much concerned with confirming and extending points of anatomy (structure) as with those of physiology (function). However, sound anatomic knowledge of the spindle is an essential prerequisite for the understanding of the function of the spindle and for the design of experiments on it.

The present anatomical picture of the spindle suggests two main questions:

1. What are the reasons for the existence of two kinds of sensory ending within the spindle and 2. Why is the spindle supplied by two different kinds of motor fiber, (for differences in structure may be presumed to be linked with differences in function)? In an attempt to answer these two questions, physiological work at present is concerned with demonstrating whether or not the two kinds of motor and sensory fibers can be found by physiological methods and if so what are their properties. Most work, thus far, has been performed exclusively on spindles in muscles of the hind limbs of the cat, but because of the similarity of the structure of muscle spindles in different muscles and in different species the results obtained may be provisionally assumed to be applicable to all spindles.

1. Physiological Study of Afferent Endings

In 1933 Matthews (73), using single fiber recording techniques, studied the responses of sensory endings to different mechanical and nervous stimuli applied to the muscle. When the muscle was stimulated to contract he recorded an increase in discharge from afferents arising in Golgi tendon organs. Since the tendon organs lie in series with the muscle fibers, they would be expected to be stretched and thus stimulated by muscular contraction. The spindles lie in parallel with the extrafusal

muscle fibers and so would be unloaded by contraction of the muscle so that the discharge of their endings would be expected to decrease and this was found to be the case.

A great deal of work has been done on the response of the primary and secondary sensory endings to dynamic and static extension of a muscle (14, 41, 45, 53, 56, 61, 73, 82). It may be concluded as a generalization from all this work that when a muscle is being stretched the muscle spindle primary endings signal both the instantaneous length of the muscle and the velocity at which it is being stretched, while the secondary endings signal mainly the instantaneous length. Another way of describing the findings is to say that the primary ending gives a large response to the dynamic component of a stimulus, over and above its response to the static component, while the secondary ending does not. This dynamic response of the primary ending is most easily studied by measuring the change in its discharge which occurs at the beginning and at the end of a period of stretching at constant velocity.

Investigators have been concerned with the question of what is the cause of the difference in dynamic sensitivity of primary and secondary endings. A reason for this was suggested by B. Matthews as far back as 1933 (73). He felt that an important part of the differentiation was caused by a variation of the mechanical viscoelastic properties of the intrafusal fibers along their length, thus causing the waveform of the stretching of the innervated parts of the intrafusal fibers to be different from that applied to the spindle as a whole. Furthermore, the initial responses of the primary ending to a sudden stretch of the muscle and that obtained

by repetitive fusimotor stimulation are different (14, 66, 75). This suggests that the adaptation of the primary ending depends on the way in which its sensory terminals are deformed rather than on the properties of the terminals themselves. The view most commonly held at present, then, is that the large dynamic response of the primary ending is caused by its lying on relatively less viscous regions of the intrafusal fibers. The dynamic response of the secondary ending is small probably because it lies on a region of intrafusal fiber with viscoelastic properties comparable to the rest of the fiber.

2. Physiological Study of Fusimotor Fibers

The small motor fibers demonstrated by the degeneration experiments of Eccles and Sherrington (91) were named "efferent gamma" fibers by Leksell (71) because their compound action potential was conducted at about the same velocity as the gamma component of the A wave of sensory fibers. Now that their function has been established they are called "fusimotor fibers", signifying that they are motor to the muscle spindles (57).

Experimental evidence suggesting that muscle spindles may be innervated by motor fibers smaller than those to the intrafusal fibers was obtained by B. H. C. Matthews in 1933 (73). He observed the response of single muscle spindle endings during contraction of the muscle elicited by stimulation of its nerve with shocks of graded strength. For some sensory endings the slowing of their discharge which occurred during submaximal contractions of the muscle was replaced by an acceleration when the stimulus was supramaximal for contraction. This effect appears to have been

best shown during repetitive stimulation, and was often found without any visible increase in the tension in the muscle on increasing the strength of a stimulus which was already supramaximal for contraction. Matthews concluded that this effect was produced by the contraction of intrafusal muscle fibers activated by nerve fibers which were smaller and had a higher electrical threshold than the large ones to the extrafusal fibers.

In 1945 Leksell (71) reasoned that if individual extrafusal fibers should happen to be regularly innervated by both large and small nerve fibers then no additional contraction would be expected on exciting the small fibers in addition to the large fibers. By blocking the large fibers in the muscle nerve, by pressure or polarization, he was able to show that tetanic stimulation of all the gamma fibers together gave less than 1% of the maximum tension the muscle could produce. He concluded that gamma motor fibers have no definite ordinary motor function.

Leksell (71) also investigated the effect of selective activation of the gamma fibers on the afferent discharge from spindles. He found that there was a considerable increase in the massed afferent discharge of the muscle on stimulating the gamma fibers alone, provided that the muscle was under some tension. He concluded that the "gamma fibers serve as regulators of sensory activity originating in the muscle." His idea that "gamma fibers may well be motor for the intrafusal muscle fibers" was firmly established 5 years later when Hunt, Kuffler, and Quilliam (55, 56, 66, 67) made a detailed analysis of the effect of stimulating functionally single gamma fibers (isolated in the ventral root) on the discharge of functionally single afferent fibers (isolated in the dorsal root). They found that stimulation of single gamma motor fibers produced no detectable tension in the muscle but did increase the discharge of single afferent

endings, which were shown to be muscle spindle endings by their behavior during contraction of the muscle; Golgi tendon organ endings were not affected. Probably the most significant conclusion derived from their work is that there are specific motor fibers to the muscle spindles, which are separate from the motor fibers to ordinary muscle fibers, and so lend themselves to independent control by the central nervous system.

A possible physiological confirmation of Boyd's (18) proposal of double motor innervation of the spindle was supplied by Jansen and Matthews (59, 60). They found from a study of the dynamic behavior of primary endings in the decerebrate cat, that different effects would be expected on exciting gamma-1 and gamma-2 fibers. Subsequently Matthews (74) found that single gamma fibers studied by this means fell into two functionally distinct groups which were provisionally called static fusimotor fibers and dynamic fusimotor fibers. Stimulation of both kinds of fibers increased the discharge of the primary ending when the muscle was at a constant length. On stretching the muscle during stimulation of static fibers the normal pronounced dynamic response of the primary ending was absent so that it behaved rather like a secondary ending. In contrast, stimulation of dynamic fusimotor fibers increased the response of the primary ending to the dynamic stimulus of stretching. The effects of the static and of the dynamic fusimotor fibers were so different that it seemed probable that this functional classification corresponded to the anatomical classification into gamma-1 and gamma-2 fibers. Unfortunately no other independent properties of the two kinds of fusimotor fibers were found which would further this suggestion and show definitely whether the dynamic fibers corresponded to gamma-1

fibers and the static fibers to gamma-2 fibers or whether the correspondence was the other way round.

As a generalization, it may be provisionally expected that activity on gamma-1 fibers (causing contraction of nuclear - bag intrafusal fibers) will excite mainly the primary endings and that activity in the gamma-2 fibers (causing contraction of nuclear-chain fibers) will excite both the primary and the secondary endings. These conclusions are arrived at by relating physiological evidence, incomplete as it is, to a rather controversial histological concept of the mammalian muscle spindle.

3. Function of Muscle Spindles

It is widely accepted that muscle spindles are not primarily important for any contribution that they make to conscious proprioception (i. e. position sense.). The main function of the muscle spindle is believed to be to play a part in the subconscious nervous control of muscular contraction, both during movement and during steady contraction.

Knowledge of the reflex effects of the afferent discharges from both primary and secondary endings is basic for an understanding of the function of the muscle spindle. The impulses from primary endings (in group I a fibers) generally excite monosynaptically the alpha motoneurons supplying their own and synergistic muscles, and inhibit those of antagonistic muscles (35). This monosynaptic reflex arc is the basis of the stretch reflex. The impulses from secondary endings (in group II fibers) excite flexor motoneurons (36).

The role of the fusimotor fibers was proposed by Kuffler and Hunt (66) who suggested that an important function of the fusimotor fibers was "to maintain the

afferent flow from the spindles in spite of a certain amount of mechanical shortening" of the spindle, so that the sensory and reflex functions of the muscle spindle could be maintained at all times. In 1951 Merton (79, 80) suggested that the muscle spindles and fusimotor fibers form part of a "follow-up length servo" by means of which the muscle can be reflexly set to any desired value. Stability is therefore maintained, for the contraction of the muscle opposes the applied tension and tends to maintain the muscle at a constant length in spite of the disturbing force. Equally, the muscle tends to maintain the same length when the load on it is reduced.

Apart from the function of the fusimotor nerves in maintaining the muscle at one length, fusimotor activity provides one way by which the muscle can be set reflexly to a variety of lengths. Any increase in fusimotor activity will tend to cause the frequency of discharge of the primary endings to increase. This will reflexly cause the muscle to shorten until the frequency of discharge of the primary endings has been reduced to its previous value (the frequency of discharge of primary endings varies with the length of the muscle) (37). Conversely, any decrease in fusimotor activity will cause the muscle to relax to a new and greater equilibrium length. On this view, the fusimotor fibers are seen as a pathway for initiating movements, by their effects on the muscle spindle, rather than as a compensator for the effects of movement upon the spindle. On present evidence, however, it is suggested (60, 81, 84) that the main function of the fusimotor fibers is to control various parameters of the servo (stiffness, damping), rather than to alter its balance point by biasing the spindle.

The servo theory also gives special meaning to the large response of the primary ending to dynamic stimuli. The primary ending of the muscle spindle,

responding to both the length of the muscle and the velocity at which it is being stretched, behaves in just the manner required to give stability to the postulated muscle servomechanism (60, 80). The primary ending may thus be said to provide damping for the stretch reflex arc, and it will thereby also counteract the tendency to oscillation produced by loading the muscle with inertia (mass). Since the dynamic response of primary endings can be varied by fusimotor activity (60, 74), this might provide a way in which the central nervous system could adjust the damping of the stretch reflex arc to suit the particular type of movement being undertaken. From a speculative point of view since, from histological evidence (18) there is a double motor supply to the spindle then it is conceivable that a dual input to the spindle provides relatively independent control of the "bias" and of the "damping" of the servo loop.

Summary of Historical Review and Aim of Project

The basis of present thinking on the role of mammalian muscle spindles in the control of movement and muscle tone is found in the work of Leksell (71), Kuffler, Hunt and Quilliam (67), Hunt and Kuffler (55, 56) and Matthews and collaborators (76). The physiological work was carried on parallel to, and became dependent on, the anatomic studies of Cooper (28), Cooper and Daniel (30), Barker (3) and Boyd (17, 18).

The anatomical work has shown that generally mammalian intrafusal muscle fibers can be divided into two morphological types: nuclear bag fibers and nuclear chain fibers. These types differ in the arrangement of the nuclei beneath the sensory endings. They also differ in their diameter, the nuclear bag fibers being the larger, and in their length, the nuclear bag fibers being the longer. Nuclear bag fibers may have a greater myofibril density than the nuclear chain fibers and do not atrophy as fast as the nuclear chain fibers following denervation (18).

The motor innervation of the two types is still controversial (5, 10, 18) but from Boyd's work the possibility exists that nuclear bag and nuclear chain fibers receive separate motor innervation, and Matthews and his collaborators have used this assumption in interpreting their results and in developing their hypothesis as to the function of static and dynamic fusimotor fibers. The scheme of Barker and co-workers of overlapping innervation could also form the basis for physiological interpretation but this has not been successfully attempted as yet.

In view of the contradictory accounts of the above authors, detailed studies

of spindle innervation in other species are desirable to further the understanding of mammalian muscle spindle innervation as a whole.

In a preliminary study conducted by Smith (92), he indicated that the muscle spindles contained in the hindpaw plantar lumbrical muscles of the rat might be a simpler model than has been established in the cat (18), rabbit (8), human (30) and opossum (63, 64). Nuclear bag and nuclear chain intrafusal muscle fibers were represented in each spindle studied. Steg (94) stated, through a personal communication with Ip, that both types of muscle fiber were present in the spindles of the lateral segmental tail muscles. Neither of these investigators, however, described any histological details of the intrafusal muscle fibers or of the sensory or motor innervation of these spindles.

Smith (92) showed cross sections of 2 spindles, one containing 3 intrafusal muscle fibers and the other 4 intrafusal fibers. This was somewhat less than that found in the cat (usually more than 8) by Boyd (18), in the opossum (9 - 10) by Jones (63) and in the human (up to 14 intrafusal fibers) by Cooper and Daniel (30).

Clearly then, the difficulty so frequently encountered in attempting to obtain a clear, precise morphological picture of all facets of the muscle spindle lies in the complexity accompanying large numbers of components in the spindle. Hopefully the rat spindle, in its simplicity, will circumvent this problem.

This project, then, is directed toward establishing, in detail, the morphology of muscle spindles contained in the rat hindpaw plantar lumbrical muscles. All facets of the spindle will be studied but the nature of the motor supply to the spindle will be stressed.

Preliminary physiological study of the lumbrical muscle nerves, the lumbrical muscles themselves, and the spindles contained therein will also be presented.

CHAPTER II

METHODS AND MATERIALS

METHODS AND MATERIALS

As this investigation progressed additional methods were employed as needed. Therefore, included in this section will be those results which pertain to the successful application, and modification, of the methods used.

A total of 130 female Wistar rats weighing 350 - 400 G. were used in this study. Of this total 96 were used for histological specimens and 34 for physiological study. In addition, the intercostal muscles from one cat were used for qualitative assessment of the silver staining technique.

I. Operative Technique

Good surgical anaesthesia, lasting $1\frac{1}{2}$ - 2 hours, was obtained with a single intraperitoneal injection of pentobarbital sodium (Nembutal). From a stock solution bottle of Nembutal (60mgm/ml.), to which 0.75 c.c. of gr. 1/100 atropine sulfate had been added, 40 mgm/kg was administered to each animal.

The animal was firmly secured on the operating table with adhesive tape. Fur was removed from the surgical site with fine hair clippers.

Clean, but not sterile, surgical technique was used. A dorsal midline incision was made extending from the spinous process of L3 to the caudal border of S1 vertebra. Laminectomy extending from L3 to S1 inclusive was made using a small single-action rongeur and small, plier-type, sharp nosed toe-nail clippers. Care was taken not to damage the spinal cord or the roots in the cauda equina. Blood loss was not excessive.

With the aid of a binocular operating microscope, and fine pointed scissors,

the dura was split in the midline extending the entire length of the laminectomy. Intermittent irrigation of the wound with warm Ringer's solution was carried out throughout the procedure.

After identification of the point of exit from the spinal canal of the fourth, fifth and sixth lumbar roots, either ventral root section or dorsal root ganglionectomy was performed. In those animals subjected to ventral root section a 2cm. segment of fourth, fifth and sixth lumbar ventral roots was excised. Dorsal root ganglionectomy was performed on these same segments by dividing the dorsal root 1 cm. proximal to its ganglion and also distal to the ganglion at the junction of the dorsal and ventral roots. Unilateral root section was performed on 60 animals. Dorsal root ganglionectomy accounted for approximately 50% of these animals.

Watertight closure of the paraspinal muscles was obtained with interrupted catgut sutures. Skin was closed with continuous silk suture. The duration of the operation was 1 - 1½ hours.

The animals tolerated the anaesthesia and operative procedure well, however the mortality rate in the first week was 50% and in the second week about 85%.

With this high mortality rate it became evident that few of the animals would survive long enough for adequate degeneration of motor or sensory axons to occur. Two possibilities were considered as to the high death rate in the first 2 postoperative weeks. Opening of the dura could have led to a bacterial meningitis. Also the stress of operation, and partial incapacitation of the rat during the postoperative period, could have unmasked a latent viral infection. Consequently, antibiotic

coverage was instituted. Starting at the time of laminectomy, and daily for 10 days postoperatively, 0.25cc of Pen Strep (STB - Div. of Canada Duphar Ltd.) containing 200,000 units penicillin and 250 mgm dihydrostreptomycin per c.c., was given by intramuscular injection. Following institution of this therapy survivors to 35 postoperative days were obtained and then sacrificed.

Post operative cannibalism of the denervated limb became a problem immediately after the animal regained consciousness. Foul-tasting solutions, such as picric acid and a mixture of alum and coal-tar, were painted on the hindlimb with no success in preventing cannibalism. As a partial solution to the problem, a Plaster-of-Paris cast was applied to the entire denervated limb with extension to the lower one-half of the body so as to restrict flexion of the trunk. This cast was applied immediately after surgery, while the animal was still anaesthetized, and required frequent reapplications during the 35 days that the animals were maintained. Animals found to have any degree of cannibalism were discarded because of retrograde degeneration in the nerve fibers.

The 35 day degeneration period was arrived at by a preliminary study in which specimens of the sciatic nerve, and various nerves in the hindfeet of normal, as well as animals subjected to root section and allowed to degenerate over 19 and 32 days, were obtained. Whole mounts of these nerve specimens were stained with osmic acid, mounted in glycerine and teased apart, using fine needles, under a binocular dissecting microscope. Myelin was found to be virtually absent at 32 days (fig. II).

To obtain nerve and muscle specimens for histological study, the hindfoot was amputated above the ankle and totally immersed in a Perspex tank containing Ringer's solution. Using a binocular dissecting microscope, fine thumb forceps and fine curved iridectomy scissors, the six plantar lumbrical muscles, with their attached nerves, were removed for histological study (fig. 1). Segments of the medial and lateral plantar nerves, as well as a portion of the sensory branch to the skin of the fifth digit, were also removed (fig. 2).

2. Histological Techniques

(a) Nerve

(i) Cross Sections

In order to determine the nerve fiber diameter distribution in the nerves to the plantar lumbrical muscles, specimens from normal, deafferented and deafferented animals were obtained. Deafferented and deafferented animals were maintained over 35 days to allow complete degeneration of the sensory or motor nerve fibers.

The animals were sacrificed and nerve specimens, as described above, were taken. The nerve trunk diameter was about 100 μ making it difficult to handle and orientate for embedding purposes. The lumbrical muscles were removed attached to their corresponding nerve thus acting as a marker.

Nerve specimens for cross section were stained and embedded as follows:

Fixation: buffered glutaraldehyde for 2 hours.

Wash: Phosphate buffer 3 or 4 times over 1 hour.

Next place in 2% osmic acid with phosphate buffer added (1:1) for

$\frac{1}{2}$ hour.

Dehydration: 70% ethyl alcohol for $\frac{1}{2}$ hour

85% " " " $\frac{1}{2}$ "

90% " " " $\frac{1}{2}$ "

95% " " " $\frac{1}{2}$ "

100% " " " $\frac{1}{2}$ "

15 minutes in propylene oxide (x2).

Embedding: Araldite II & propylene oxide (1:1) for 3 hours

Araldite II only for 12 hours

Araldite II was made up as follows:

10ml. Araldite

10ml. Araldite Hardener

1ml. Dibutyl Phthalate

0.4ml. Accelerator

The nerve specimens were carefully aligned in the center of grooves, measuring 2mm x 5mm x 18mm, which were milled in the surface of a sheet of Teflon. Araldite II was dropped in to enclose each of the nerve specimens and the entire sheet placed in an oven to harden over 36 hours. Nerve specimens were thus accurately aligned in the longitudinal axis of the Araldite pellet.

Cross sections about 0.5μ thick were cut by a glass knife in a Porter - Blum MT - 2 ultramicrotome. The cross sections were placed on a glass microscope slide and allowed to dry. Sections were studied with a light microscope with an oil immersion objective. No cover-slip was used. No counter-staining was necessary.

Half micron thick cross sections were cut from the Araldite-embedded nerves in order to obtain reliable measurements of nerve fiber diameters. The smallest myelinated nerves were about 1μ in diameter, thus the oil immersion objective (100X, N. A. 1.30) had to be used which, according to the

manufacturer's data sheet, had a lateral resolving power of $0.3\ \mu$. Sections of conventional thickness, say $5\ \mu$, would have prejudiced the accuracy of the measurements. The sections would have contained segments of axons $1\ \mu$ in diameter and $5\ \mu$ long and these may or may not have been perpendicular to the plane of section. Since the depth of field of the 100 X objective was only $0.63\ \mu$ a poor quality image would have resulted from the out-of-focus parts of the specimen. For $0.5\ \mu$ sections, then, optimal lateral and axial resolution was obtained using 10X eyepieces and 100/1.30 objectives.

(ii) Photography and Measurements

A Nikon light microscope model SIR -Ke mounted with a Nikon Microflex Model EFM semiautomatic photographic attachment was used for all photomicrography.

All measurements of axon diameters were taken from enlarged prints of photomicrographs. The danger of alteration of the true axon diameters by varying the intensity of light and/or the exposure time was considered, therefore a study similar to that conducted by Williams and Wendell-Smith (98) was undertaken. A single nerve cross section was chosen and examined, without a coverslip, using an oil immersion objective. Two measurements, at right angles, of each axon were taken directly with an eyepiece micrometer sensitive to $0.5\ \mu$. The same cross section was then photographed using fixed illumination but varying the time exposure to both extremes above and below the zero point. The 35mm. negatives were then enlarged to give a total magnification of 2.5 thousand times and 2 measurements, again at right angles, were taken of each axon with a vernier caliper. It was found that values obtained from individual axons were within 5%, using the two methods, if the exposure time was set to that required to

give a normal negative, after normal development over the time recommended for the emulsion used.

A representative section of each of the nerves studied was photographed, as outlined, on Kodak Plus-X black and white 35mm. film and then enlarged with a Leitz Focomat Enlarger on 5" x 7" single weight glossy Koda-bromide F-4 or F-5 photographic paper. Total magnification was 2.5 thousand times.

Nerve fiber diameters were measured from the photographic enlargements using a sheet of Perspex inscribed with circles corresponding to $1\ \mu$ intervals at 2.5 thousand times magnification. Nerve fiber diameter histograms were then constructed by placing the axons in $1\ \mu$ groupings.

(iii) Controls

Concurrent control on the adequacy of root section were carried out. A portion of the sensory skin nerve to the fifth digit was stained, as a whole mount, with osmic acid and teased apart with fine needles. Myelin was absent in animals which had dorsal root ganglionectomy. Cross sections of these nerves, embedded in Araldite, were studied for degeneration. Specimens from animals subjected to ventral root section and dorsal root ganglionectomy were obtained.

After staining with osmic acid, the lumbrical muscle markers were divided from their nerve trunks and squashed flat between two slides. Under a dissecting microscope, and using two 20 guage needles, spindles were teased out to show the presence or absence of sensory axons in deafferented and deafferented animals.

An additional control on the completeness of ventral root section by electrical

stimulation of the medial and lateral plantar nerves showed no visible muscle contraction as observed with a binocular dissecting microscope. This would not, of course, rule out the presence of fusimotor fibers.

Deafferented nerve trunks contained few nerve fibers, most of which measured 1-2 μ . In order to be sure that myelinated motor axons beyond the resolution of the light microscope were not present, some thin sections (silver) were cut from the nerve trunk to lumbrical muscle 5 as this appeared to be the most difficult of the nerves on which to obtain good resolution. These sections were mounted on copper grids having a carbon support film, and examined in a Philips EM 100 electron microscope at an accelerating voltage of 60 KV.

(b) Muscle

(i) Cross Sections

Normal lumbrical muscle specimens were fixed relaxed in buffered glutaraldehyde, embedded in paraffin and stained with hematoxylin and eosin. Ten percent formalin was tried as a fixative but excessive distortion of extrafusal and intrafusal muscle fibers occurred. Serial cross sections 7 μ thick were cut. Intrafusal fibers were traced through each of the muscles sectioned and two measurements, at right angles, were taken from each fiber. Every tenth cross section was measured and plotted except where more detail was needed, as in the central nuclear region, where all the sections were examined. Measurements to 1 μ were obtained with an eyepiece micrometer using a 40 X objective. Fourteen complete spindles were reconstructed. The length and diameter of the capsule was also noted.

A comparable muscle was fixed in situ with glutaraldehyde, stained and embedded as described for the nerve cross sections and $1\text{ }\mu$ thick cross sections through the equator of the muscle were obtained. These sections presumably contained all of the fibers traversing the length of the muscle. The best section was photographed and enlarged to 250X on photographic paper. Two measurements, taken at right angles, were made of each extrafusal muscle fiber using a vernier caliper and an extrafusal muscle fiber diameter histogram constructed. For an estimation of shrinkage in paraffin-embedded material, the mean of the distribution histogram was compared to the mean of a diameter distribution obtained in a similar way from a cross section of a comparable lumbrical muscle fixed relaxed, embedded in paraffin and stained with hematoxylin and eosin.

(ii) Whole Mounts

The wet weights of all six lumbrical muscles were obtained using a sensitive weight scale. Whole mounts were then stained with Cole's gold chloride method (27) and squashed between 2 slides. Under the binocular dissecting microscope, and using transmitted light, the number and location of the spindles in each of the muscles could be determined by observing their capsules. Measurements of spindle length were made, by an eyepiece micrometer mounted on the dissection microscope, from unteased muscle spindles as well as from 76 teased-out spindles. A spindle length histogram was constructed and compared to spindle length measurements obtained from the serial reconstructions of spindles from the paraffin-embedded material.

In order to determine the details of motor and sensory innervation of the spindles in whole mounts of the muscles under study, several staining techniques were

employed. The gold chloride methods of Cole (27) and Boyd (16) were found to be unsatisfactory because of inadequate staining of the myelin covered axon, and spotty, coarse staining of fine, terminal unmyelinated axons though the endplates stained well. Boyd's methylene blue perfusion technique (15) proved to be inadequate because of inconsistent staining and failure to show fine details of the innervation. The Gros-Bielchowsky silver method (100) was abandoned because of lack of axon staining within the spindle.

A silver stain method described by Winkelmann (99) gave the best staining of thick and fine myelinated and unmyelinated axons in whole mounts. One hundred and twenty spindles were teased out of normal lumbrical muscles, as well as lumbrical muscles from animals which had undergone dorsal root ganglionectomy and allowed to degenerate over 7 days. Sensory endings degenerate over this short period of time (18). Teasing was accomplished, under a binocular dissecting microscope, with 2 fine needles and efforts were made to preserve the entire spindle, with its innervation, as much as possible. The polar ends of the spindle, being thin and fragile, were easily broken off in the teasing. The teased spindles were mounted in glycerine on a microscope slide and covered with a cover-slip. Several microscope objectives, ranging from X4 - X40, were used to examine the mounts. Photographs were taken, as described for the nerve cross sections, of intact and crushed specimens. Crushing between the cover-slip and slide was necessary, at times, to separate the intrafusal muscle fibers for clarification of the innervation and identification of the individual intrafusal muscle fibers. The photographing and enlarging process was similar to that described for nerve cross sections. Some loss of fine detail through the various

photographic stages was noted but this was not considered to be too significant.

Selected enlargements were labelled and mounted on manilla blanks and the entire plate re-photographed. Montages were made to show details occurring at different focal planes. Limited retouching of fine terminal axons, for better contrast, was necessary to restore fine details as seen on the original negative.

Measurements of motor and sensory axons 1mm. from the spindle were taken from the enlarged prints with a vernier caliper. Measurements of nuclei contained in the nuclear bag and nuclear chain muscle fibers were also taken from the photographic enlargements.

Cat muscle spindles stained with silver have been studied histologically (4). As a qualitative check on the staining technique used, whole mounts of cat intercostal muscles were stained with the Winkelmann silver technique (99) and 30 spindles examined.

(3) Physiological Techniques

A limited study of the properties of lumbrical muscle 5 and its nerve was carried out in order to test some of the implications of the histological results.

(a) Nerve Conduction Studies

Compound sensory or motor action current recordings were made by stimulating spinal roots and recording from the nerve to lumbrical muscle 5. The temperature of the animal and the conduction distance were measured in each of five experiments. Fastest conduction velocities for sensory and motor fibers were calculated.

Eleven experiments were carried out in which the velocities were measured in nerves kept ex vivo in a Ringer solution. In these latter experiments the chamber shown in figure 17 was used. The recording chamber consisted of two blocks of

Perspex cemented together. The lower block had a milled zig-zag groove through which temperature - controlled water was pumped. The upper block contained a central longitudinal groove which, when filled with Ringer solution, provided a temperature controlled environment for the nerve. Seven to eight cm. of nerve was placed in the groove and across moveable partitions in the groove (see fig. 17). Where the nerve crossed the partitions it was covered with Vaseline to form a high external resistance so that current could be injected into the nerve in order to stimulate it. Also current flow could be detected, across the high external resistance created by the Vaseline, for recording purposes.

The stimulating and recording apparatus was connected as shown schematically in figure 17. The stimulator was a Devices stimulator which delivered rectangular pulses of variable amplitude and duration. The duration of the stimulating pulses used in these experiments was 0.2 msec. The stimulating electrodes of silver chloride coated silver wire could be connected either across partition (1) or partition (2) so that a difference measurement could be obtained for calculation or conduction velocities. Recording electrodes, which were also Ag-AgCl junctions, led from the right hand partition shown in figure 17 to a Grass P8AC preamplifier and then to a Tektronix 565 oscilloscope. The compound action potentials were displayed together with 1 msec time-markers and photographed with a Grass Kymograph camera.

(b) Measurements of Muscle Contraction

Muscle contraction was also measured ex vivo in the same temperature - controlled chamber with a slight modification shown schematically in the inset (B) of figure 17. The muscle was held by its tendons between a split Nylon rod, in the wall of the chamber, and a strain guage myograph which was mounted on a manipulator.

The output of the myograph, which consisted of bonded strain gauges cemented on a piece of razor blade and excited by a Tektronix 3C66 amplifier, was displayed on the oscilloscope. Loading of 100mgm on the strain gauge gave a deflection of 1cm. on the oscilloscope. In the seven experiments conducted with this arrangement several were performed with combined nerve and muscle recording amplifiers so that the afferent nervous activity could be observed on extension of the muscle.

The same physiological solution was used throughout. It was similar to that described by Liley (72) and had the composition (mM): NaCl, 138.0; NaHCO_3 , 12.0; KCL, 5.0; MgCL_2 , 1.0; CaCl_2 , 2.0; KH_2PO_4 , 1.0. This was mixed fresh each day from stock solutions. Immediately before an experiment 1G/L dextrose was added and the solution saturated with a mixture of 95% O_2 and 5% CO_2 .

CHAPTER II

The first part of the study was a review of the literature on the topic of the study. The review was conducted in order to determine the current state of knowledge on the topic and to identify the gaps in the literature. The review was conducted in a systematic manner, using a search strategy that included a search of the literature in the field of the study. The review was conducted in a systematic manner, using a search strategy that included a search of the literature in the field of the study.

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CHAPTER III

RESULTS

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RESULTS

(I) Gross Anatomy of the Nerve Supply to the Hind Foot

A well illustrated description of the innervation of the rat hindlimb has been compiled by Greene (44). However because important details concerning the innervation of the lumbrical muscles were lacking, a detailed dissection of important nerve trunks was carried out. Electrical stimulation of spinal roots and nerves and observation of muscle contraction was used to check motor pathways.

There are six plantar lumbrical muscles (fig. 1) in the hindpaw of the rat. The four large muscles arise from the ventral surface of the tendon of flexor hallicus longus where it divides into slips for digits 2-5. A well developed, but thin, tendon is present at the distal ends of these muscles by which they insert on the proximal end of the first phalanx of digits 2, 3, 4, and 5. These lumbrical muscles henceforth will be identified by the number of the digit on which they insert.

Two smaller lumbrical muscles were found to arise from the ventral surface of the tendon of flexor hallicus longus before it divides, and insert on the preaxial side of flexor digitorum brevis tendons to digits 3 and 5. These two slips, by virtue of their central location on the plantar surface of the foot, will be referred to as medial-central (M.C.) and lateral-central (L.C.) lumbrical muscles.

All muscles in the hindfoot receive innervation from the medial and lateral plantar nerves which are formed by the division of the tibial nerve at its termination (fig. 2). With careful dissection the extremely fine nerves entering the equatorial region of each of these muscles can be found. Fine muscular branches can be traced from the medial plantar nerve to the medial and lateral central lumbrical muscles. The fine

fine muscle nerves to the remaining lumbrical muscles can be found lying on the ventral surface of the interossei. A sensory branch of the lateral plantar nerve to the skin of the 5th digit could be easily found and was used in a control procedure for the completeness of deafferentation (fig. 2).

While observing for muscular contraction with a binocular dissecting microscope, electrical stimulation of the medial and lateral plantar nerves revealed that the usual motor innervation to lumbrical muscles 2, 3, 4 as well as medial and lateral central muscles to be from the medial plantar nerve whereas the fifth lumbrical muscle received motor innervation exclusively from the lateral plantar nerve. Occasionally the fourth lumbrical muscle had motor innervation from both medial and lateral plantar nerves (fig. 2). The above description of the lumbrical muscle innervation is at variance with Greene's concept (44).

There are six lumbar nerve roots in the rat but only the fourth, fifth and sixth roots contribute to form the sacral plexus (fig. 2). Each of the spinal roots in the cauda equina exists as a separate long fine single filament. Because of the narrow intraspinal canal, the dorsal and ventral roots of each segment maintain a relatively constant relationship to each other up to their junction at the intervertebral foramen. Just proximal to the intervertebral foramen the characteristic swelling of the dorsal root ganglion is evident. A single filament measuring 0.5-1mm joins the distal border of the dorsal root ganglion to the ventral root at the intervertebral foramen.

The sciatic nerve, taking origin from the sacral plexus, innervates various muscles in the thigh then divides at the popliteal fossa into its two terminal components, the tibial and the common peroneal nerves (fig. 2). The tibial nerve takes a deep course

between the two heads of gastrocnemius, where it gives off muscular branches, and continues distally to divide just above the ankle into medial and lateral plantar nerves. Before entering the plantar surface of the foot, an anastomotic branch joins these two nerves.

Electrical stimulation of the fourth ventral root gave rise to contractions of muscles at the hip. Stimulation of the fifth lumbar root resulted in contraction of muscles in the thigh and calf and stimulation of the sixth root resulted in contraction of intrinsic muscles of the foot as well as excitation of the peroneal muscles. The first sacral root did not have any motor component in the hindlimb but did appear to innervate proximal muscles of the tail.

(2) Morphology of Muscle Spindles

All measurements given in the results are not corrected for shrinkage unless otherwise indicated.

(a) Numbers and distribution of receptors

All lumbrical muscles studied showed a tendency for the muscle spindles to be concentrated in the middle third of the muscle near the region of nerve entry (fig. 1). Two Golgi tendon organs which were found were situated at the distal musculotendinous junction of lumbrical 2.

The total number of spindles (table I) varied little from muscle to muscle in the same and in different animals. The greatest number of spindles seen in six lumbrical muscles varied from 3 to 8. The larger lumbrical muscles (2, 3, 4, 5) consistently contained more spindles than the smaller muscles (MC, LC) (table I). No tandem spindles were found (figs. 1, 3).

(b) Structure of muscle spindles

(i) The spindle capsule

The spindle was surrounded in its central zone by a cellular and connective tissue capsule which had two clearly distinguishable components, an outer sheath or capsule (fig. 4) which enclosed the periaxial lymph space and was continuous with the sheath of Henle on the incoming nerve, and a thin inner sheath or axial capsule which closely invested the intrafusal muscle fibers and their associated sensory endings (fig. 5 (B)).

In the central region some of the inner layers of the outer capsule could frequently be seen to diverge from the remainder and to cross the periaxial capsular space obliquely, forming one or more subcompartments within it (fig. 4 (B) (C)). These contain myelinated and non-myelinated nerve fibers in the course of their oblique passage through the capsule wall. The thickness of the outer capsule diminished toward the poles of the spindle until the intrafusal fibers eventually passed beyond the final single layer to enter the general endomysial tissue space (fig. 4 (A) (F)).

The widest diameter of the capsule in the equatorial region varied from 60 μ to 90 μ . The spindle capsule varied from 200 μ to 1000 μ in length and was one third to one quarter of the total length of the spindle. The average length in 14 spindles was 440 μ .

The axial capsule was much thinner than the outer capsule (fig. 5 (B)) and was seen to form more or less complete envelopes around each of the intrafusal muscle fibers at the equator of the spindle. Away from the equator these coverings became less complete.

(ii) Number of intrafusal fibers

Each spindle capsule contained from 3 to 5 intrafusal fibers, the average number in 14 capsules being 3.5. Most spindle capsules contained two nuclear bag and two nuclear chain fibers (fig. 3). One spindle (fig. 3 spindle III) did not contain any nuclear bag fibers. Only three intrafusal fibers were present. The largest fiber, containing only a single axial row of nuclei at its equator, was comparable to intermediate fibers described by Jones (63) and was therefore regarded as a nuclear bag fiber.

(iii) Diameter of intrafusal fibers

Diameters of intrafusal fibers were measured at one pole of the capsule (c.f. fig. 3, spindle I, line b). The diameters of both nuclear bag and nuclear chain fibers varied somewhat along their length (fig. 3). There was some overlap of the distribution at the level of the capsule pole (fig. 7). The mean diameter for the nuclear chain fibers was 7.7μ and for the nuclear bag fibers 10.0μ . Corrections for 29% shrinkage (fig. 6) resulted in means of 12.4μ and 16.1μ respectively. The mean diameter of the extrafusal muscle fibers (corrected 30.7μ) was two to three times that of the intrafusal fibers (fig. 7).

(iv) Spindle Length

The length of spindle whole mounts stained with gold chloride ranged from 1 to 6.5mm with an average value in 44 spindles of 3.32mm (fig. 8). Total length of 14 spindles reconstructed from serial cross sections ranged from 0.5 to 3.0mm with a mean of 1.5mm (fig. 8). Assuming that insignificant shrinkage occurs in the gold chloride stained specimens then a comparison of the two means indicates a shrinkage of 55% in the specimens stained with hematoxylin and eosin. This

shrinkage was somewhat greater than that quoted by Boyd (18) and was probably incorrect because of the relatively smaller number of spindles reconstructed as compared to the gold chloride stained whole mounts. The error in shrinkage was further accentuated by the lack of spindles, reconstructed from serial cross section, approaching the length of 6.5mm seen in the gold chloride specimens. Using Boyd's (18) 40% for shrinkage correction, a replot of the original graph (fig. 8) would show that the length of the spindles reconstructed from serial cross sections would overlap completely a similar histogram of spindles stained with gold chloride.

Typical nuclear bags and nuclear chains, each flanked by a characteristic myotube region, were present at the equators of most spindles (fig. 3). Nuclear bag fibers had an average length of 1.58 mm (fig. 9) with about one-third of their length projecting usually symmetrically beyond each pole of the capsule.

Nuclear chain fibers had an average length of 1.20mm (fig. 9) and varied in their relations to the poles of the spindle capsule (fig. 3): a few began and ended at the capsule poles; some began near one pole and extended well beyond the opposite pole, often as far as the accompanying nuclear bag fibers; the majority extended beyond the poles symmetrically and were usually slightly shorter than the accompanying nuclear bag fibers (fig. 9). The extracapsular portions of most spindles, therefore, contained both nuclear bag and nuclear chain fibers. No branching of intrafusal fibers was seen and this appears to be unique to the rat.

(v) Nuclear characteristics

The equatorial region of the spindle contained the nucleated portion of the intrafusal fibers. The nuclear bags were 100 - 400 μ long and the myotubes 200 - 700 μ

long with an overall mean in 22 fibers of 489μ which constituted one third of the nuclear bag fiber length.

The nuclear chains and their flanking myotubes together measured from $100 - 700\mu$ in length with a mean of 310μ in 25 fibers. This constituted one quarter of the nuclear chain fiber length. The nucleated portions of both nuclear bag and nuclear chain fibers lie largely within the spindle capsule which had a mean length of 439μ .

The constituent nuclei of the nuclear bag fibers were large and granular (fig. 5 (B)) and the numbers in any one cross section of a single nuclear bag ranged from 2 to 3 (fig. 3). The nuclear shape varied with location in the nuclear bag fiber (figs. 10, 28 (A)). Nuclei found in the bag region were more-or-less round in shape measuring 4 to 6μ in length and 4 to 6μ in width. In the myotube region of the nuclear bag fiber the nuclear shape became progressively elongated from the bag to the capsule pole as indicated by the progressive increase in nuclear length from 5 to 15μ (fig. 10).

Only one nucleus was seen in any one cross section of a nuclear chain fiber. The constituent granular nuclei were 3 to 5μ in diameter and only partially filled the fiber which had a mean diameter of 7.7μ . The nuclei became progressively elongated, varying from 8 to 16μ in length, as the capsule pole was approached (figs. 10, 28 (A)).

Nuclear shape characteristics, as determined in the cat (fig. 10), generally paralleled that as outlined for the rat. The nuclei of the cat spindles were generally larger in length and width than those in the rat. It is provisionally concluded that the difference in nuclear shape in nuclear bag and nuclear chain muscle fibers is a general characteristic of mammalian muscle spindles.

(3) Composition of the Nerve Trunk to the Lumbrical Muscles

(a) Controls

Determination of the motor and sensory content in a peripheral nerve required well-controlled degeneration experiments. Previous investigators (1, 17) performed serial cross sections of the distal dorsal root stump to determine whether any ganglion cells remained. However, in those animals which had a few ganglion cells remaining after dorsal root ganglionectomy additional control procedures, similar to those described for this study, showed that fibers from the persisting neurons did not innervate the particular muscles under study. Because of lack of specificity as to innervation, serial transverse sections of the distal dorsal root stump were not carried out. The control procedures which were carried out, however, revealed absence of sensory fibers at the spindle level (fig. 12 (A)) and in the sensory skin nerve (fig. 13 (D)) in animals which had dorsal root ganglionectomy. The converse was true for the spindle innervation (fig. 12 (B)) and sensory skin nerve fiber content (fig. 13 (C)) in animals which had ventral root sections.

During the course of the degeneration experiments, an investigation (40) appeared which showed evidence for segmental demyelination of nerves in the feet of guinea pigs. This pathology presumably resulted from pressure points created by the wire cages in which these animals were housed. The rats in this study were similarly caged. Several normal adult rats were sacrificed and several superficial and deep nerves in the hindfeet were stained with osmic acid and teased apart with fine needles. No evidence of nerve degeneration or segmental demyelination was found.

(b) Muscle nerve composition

Cross sections of the nerve to lumbrical 5 showed the normal intact nerve (fig. 13 (A)), the sensory fiber content (fig. 13 (B)) and the motor fiber content (fig. 14 (B)). The electron-micrograph (fig. 14 (A)) confirmed that all of the smallest myelinated motor nerve fibers present could be resolved with the light microscope. These cross sections represent the general trend as seen in the nerve trunks to the remaining five lumbrical muscles.

(i) Normal nerve content

The normal intact nerve to lumbrical muscle 5 showed several large and small axons (fig. 13 (A)) with a distribution which was essentially bimodal (fig. 15 (a)). A similar bimodal distribution was also seen in the normal nerve trunks to lumbrical muscles 2, 3 and 4 (fig. 15 (a)(b)). Their counterparts to LC and MC lumbrical muscles showed a trimodal distribution (fig. 15 (a)).

(ii) Afferent fiber content

The cross section showing only sensory fibers (fig. 13 (B)) revealed a predominance of large fibers with a bimodal distribution (fig. 15 (b)). The sensory fiber distribution to the other lumbrical muscles was also bimodal (fig. 15 (a) (b)) except for LC lumbrical muscle nerve which was trimodal. Note that the largest nerve fibers (10μ) found in the normal nerve trunk were also present in the histograms of the sensory nerve fiber distributions.

(iii) Efferent fiber content

The efferent fibers to lumbrical muscle 5 were small (Fig. 14 (B)) and few in number (table I). This general trend persisted in all of the nerves to the lumbrical muscles and was consistent from animal to animal. A bimodal distribution was evident

in the nerve trunk to lumbrical muscles 3,4 and 5, whereas LC, MC and 2 had a trimodal distribution (fig. 15 (a) (b)). Only a third of the total nerve fiber content in each of the nerve trunks was motor (table 1).

The largest motor fibers in all muscle nerve trunks were 7 - 8 μ (fig. 15 (a) (b)). A bimodal distribution of motor fibers in lumbar ventral roots 4, 5, 6 was present (figs. 14 (C), 16) with one peak (4 μ) lying between 1 μ and 8 μ and the second peak (14 μ) lying between 9 μ and 16 μ . All of the motor fibers (alpha and gamma) to the lumbrical muscles must lie within the first peak. This was supported by stimulating lumbar 4,5, 6 ventral roots with graded stimulus strengths. At threshold, only the proximal muscles in the hindlimb contracted indicating that their innervation was by large motor axons of low threshold. The lumbrical muscles contracted only after the stimulus strength had been somewhat increased. This ruled out motor innervation of the lumbricals by branches of larger axons which were innervating the larger proximal muscles.

(C) Physiological characteristics of a lumbrical nerve trunk

All recordings were made from the nerve to lumbrical muscle 5 because this nerve could be removed intact from the animal with little or no damage and because in a preliminary study, which showed that the nerve supply to the foot originated from the lumbar roots 4, 5 and 6, it was evident that in most animals the entire motor and sensory supply to this muscle was contained in the sixth lumbar root. Conduction velocities were measured in vivo and ex vivo.

The compound action potential (fig. 18 (D)) had a similar configuration to the histogram of the normal muscle nerve to lumbrical 5 (fig. 15 (b)). The conduction velocity distribution of sensory (fig. 18 (E)) and motor (fig. 18 (F))

fibers also corresponded to their histograms. The leading edge of the sensory fiber action potential was faster than that of the motor. This conforms to the maximum fiber diameters found in the sensory and motor histograms.

The temperature dependence of the conduction velocity was determined by varying the temperature between 15°C and 35°C (fig. 18 (A)(B)(C) and graph). Wavefront measurements were made (fig. 18 (1a)), however corrections for the "dead time", which is the time required for depolarization of nerve to threshold before a nerve action potential could be propagated, were necessary. This was achieved by stimulating the same nerve at two different sites (fig. 17 (A)) and calculating the conduction velocity over the segment between the two stimulating sites. A dead time of 0.5 msec. was obtained and appropriate corrections to the conduction velocities made. The wavefront measurement of the compound action potential showed a conduction velocity-temperature dependence of 1M/sec for each 1°C change, between 15°C and 35°C, for the largest nerve fiber groups (fig. 18 (1a)(1)). The two peaks made up of smaller nerve fiber groups, (fig. 18 (II)(III)) did not show this temperature dependence.

Conduction velocities up to 50m/sec. at 35°C were obtained in some specimens. The fastest conduction velocities at 30°C ranged between 35 and 43.2 m/sec., these being the larger sensory fibers. It was felt that the rat usually conducted in this range, from the spinal cord to the hindpaw, since the mean rectal temperature in 20 rats was 35.9 (± 0.79)°C and the mean temperature as measured on the plantar surface of the hindpaws in 38 animals was 27.3 (± 0.93)°C.

Conduction velocities of sensory and motor fibers in vivo at 29°C to 30°C fell along the regression lines (fig. 18) plotted for conduction velocities as measured on nerve specimens in the recording chamber (fig. 17 (A)). The immediate indication from the comparable conduction velocities was that no appreciable damage was inflicted on the nerve during removal from the animal. It was also evident that the faster conducting sensory fibers fell within the first peak of the compound action potential and that the motor fibers were contained in the slower portion of the first peak and in the second peak (fig. 18).

An indication of the minimal number of motor units contained in lumbrical muscle 5 was obtained (fig. 19 (A)). In seven animals studied, a minimum of 3 to 5 motor units were present. These motor units were noted to have a high fusion frequency, fusing at 100 imp/sec. (fig. 19 (B)).

Each animal studied, consistently had few motor axons (5 to 9) in their nerve trunks to the lumbrical muscles. The total number of motor axons approximately equalled the total number of spindles contained in each of the lumbrical muscles (table I). With 3 to 5 motor units present one must assume that extensive branching of motor axons, within the lumbrical muscle to innervate all the spindles, was occurring.

(4) The Motor Innervation of Muscle Spindles

(a) Mixed Innervation

Six specimens showing skeletofusimotor, or beta innervation, of the muscle spindles were teased out intact (fig. 20). In each case a single motor axon could be easily traced to its bifurcation. One branch was followed to its termination on

extrafusal muscle fiber end-plates. The axon branch to the spindle always terminated on an end plate on a nuclear bag fiber in the midpolar region of the spindle. No additional motor innervation to this fiber was present. The nuclear chain fibers in these spindles received pure fusimotor innervation.

Fusimotor axon branching to innervate adjacent spindles also occurred (fig. 21). In this case the fusimotor axon entered the capsule of one spindle from where it passed to one polar end and terminated on a nuclear bag fiber by two motor end plates. Just prior to termination, it gave off a branch which ended on the intracapsular portion of a nuclear bag fiber by a motor end plate.

(b) Structure and distribution of motor nerve endings

The controversy which exists as to the motor innervation of the individual intrafusal fibers appeared to stem, in part, from the varying results obtained using different staining techniques. The results presented here are those obtained from examining silver stained specimens. In order to estimate staining quality, several spindles from the intercostal muscles of a cat (fig. 22) were stained with the same silver technique as used in the rat and compared with spindles stained by Barker's technique (4, 9). The two staining techniques were comparable in staining quality of spindle whole mounts.

(i) Plate endings

In silver stained preparations the motor plate-endings appeared as discrete round to oval structures on the striated poles of the intrafusal fibers (figs. 23, 24). They were similar in structure to extrafusal motor end-plates (fig. 23 (A)(B)) but were usually more oval and smaller. A typical extrafusal motor end plate measured

4 μ by 6 μ ; the usual intrafusal plate-ending was 3 μ by 4.5 μ and covered only part of the width of the intrafusal muscle fiber.

This type of motor end-plate was found mainly on nuclear bag fibers beyond the pole of the spindle capsule, however it was occasionally also found on the extracapsular portion of the nuclear chain fibers (figs. 23, 24).

(ii) Filamentous endings

The motor endings found on nuclear chain fibers and described as "diffuse multi-terminal endings" by Hess (46), "gamma-2 network" by Boyd (17, 18), and "trail endings" by Barker and Ip (10) were not present in the rat. The nuclear chain motor ending commonly observed was of a filamentous nature whereby the motor axon would taper to form a fine strand just prior to its termination (fig. 24 (B) (F)). This form of motor termination was also noted to exist occasionally on nuclear bag fibers (fig. 24 (D) (E)). Occasionally one muscle fiber had both types of ending (fig. 24 (D)). Branches of a fusimotor axon to two separate nuclear chain fibers were noted to end as plate and filamentous endings (fig. 24 (B)). Several combinations of motor endings on nuclear bag and nuclear chain fibers are shown schematically (fig. 26(a) and (b)).

(C) Motor nerve fibers ending within the spindle

(i) Diameters and numbers of spindle motor nerves

Spindle motor nerve fibers were measured and counted 1mm from individual spindles in silver stained preparations. All spindles received a thick axon, which lay in the 1.5 to 2 μ range, and a thin axon measuring 0.5 to 1 μ .

Motor nerve fibers most frequently entered spindles, lying at the proximal or distal ends of the muscle, at the spindle pole which lay closest to the muscle equator

(fig. 30(C)). The fusimotor fibers would then terminate on intrafusal muscle fibers at this end. Additional motor innervation of the opposite polar end of spindle was only rarely seen. Occasionally the motor axons would enter the spindle in company with the sensory axons at the capsule and would innervate either end of the spindle.

Spindles lying in the middle one third of the muscle had motor innervation at both spindle poles more frequently than spindles lying in the distal or proximal one third of the muscle. Motor fibers usually entered these spindles in company with the sensory axons at the capsule (figs. 27 (B), 30 (A)) and innervated either end or occasionally both ends of the spindle.

(ii) Mode of termination of spindle motor nerve fibers

Each spindle received 2 motor axons - a thick and a thin one (figs. 25, 26 (a) and (b)). The difference in axon diameter did not persist after the nerve fibers had entered the spindle (fig. 25). While in the spindle they were much the same size and at times it was difficult to be sure of their origin.

Branches of the thick axon usually innervated nuclear bag fibers only, whereas branches of the thin axon innervated nuclear chain fibers (fig. 26 (a) (b)). Infrequently it was noted that the converse was true. That is, the thick axon would innervate nuclear chain fibers and the thin axon the nuclear bag fibers. No specificity existed as to the type of ending associated with either the thick or thin axon, however the thick axon usually terminated on a plate ending and the thin axon on a filamentous ending (fig. 26 (a) (b)). No specimen showed overlapping innervation of nuclear bag and nuclear chain fibers from a motor axon.

(5) The Sensory Innervation

(a) The Primary ending and its nerve

The group Ia afferent nerve fibers, whose total diameter 1mm from the spindle varied from 8 to 11 μ (average 9.8 μ) in osmic acid preparations, entered the capsule more or less at right angles, dividing once or twice dichotomously as it did so (fig. 27(A) (C)). Within the capsular space, each branch divided once or twice more. Many of these branches ended in a spray of nonmyelinated branches, others lost their myelin sheath and passed directly without further branching to their terminations. One or more of the fine nonmyelinated terminal branches passed to each intrafusal muscle fiber in the region of its central nuclear aggregation. These fine branches became continuous with the primary sensory nerve ending which usually took the form of a series of tightly wound regular spirals (fig. 28). The nuclear chain spirals generally had a greater pitch and appeared to have fewer turns than that found on the nuclear bag fibers (fig. 28 (C) (D)).

The primary ending occupied the equatorial region only. The average total length of the primary ending was 176 μ with a range of 130 to 240 μ . It therefore occupied about one eighth of the total spindle length. One third to one half of the intracapsular portion of the intrafusal fibers was covered by primary endings. No spindle contained more than one primary ending. Two simple spindles were found sharing a common group Ia afferent fiber (fig. 29).

(b) The secondary ending and its nerve

The secondary sensory ending was present in about 50% of spindles. The group II afferent nerve fiber which terminated in these endings had a total diameter, in osmic acid stained preparations, which varied from 6 to 10 μ with a mean of 7.8 μ .

The group 1a and group 11 afferent nerves to the spindles were derived from the same small intramuscular nerve trunk and entered the spindle capsule together (fig. 27).

The endings of the fine terminal branches of the group 11 afferent fiber occupied chiefly the juxta-equatorial portion of all the nuclear chain fibers. A small part of the secondary ending did however, lie on the myotube region of the nuclear bag fibers. The average total length of the secondary ending was 147μ with a range of 100 to 260μ . It thus covered one third of the intracapsular portion of the intrafusal fibers.

The number of secondary endings per spindle varied from 0 to 2, 0 to 1 being the commonest. If two were present they almost invariably lay on different sides of the primary ending; only rarely were two secondary endings seen on the same side of the primary. If two secondary endings were present in a spindle they were usually supplied by nerve fibers that were separate as far back as they could be traced. No single group 11 afferent fiber supplied secondary sensory endings in more than one muscle spindle.

(c) Other nerve fibers in the spindle

Infrequently, very fine (0.5μ) unmyelinated nerve fibers were seen ramifying quite widely through the muscle spindle capsule (fig. 30 (B)). These nerve fibers never came in contact with the intrafusal muscle fibers. Their origin was not ascertained, however with the presence of blood vessels in the capsule (fig. 30 (A)) an autonomic source must be considered.

(6) Physiological Characteristics of the Muscle Spindle

Preliminary physiological study of the spindle was carried out by recording from spindle afferent nerve fibers. Stretching the muscle 1mm resulted in an increase in afferent discharge from the spindle (fig. 3I (B)). The graph in figure 3I shows the response of the spindle to rapid stretch of the muscle. The peak discharge of the dynamic response to the stretch was reached in about 60 msec. and then fell over 110 to 120 msec. to the static discharge level, during which time the muscle extension was maintained. The static response to the maintained stretch was sustained for several hundred milliseconds.

Figure 3I (A) shows the recorded afferent response to the addition of succinylcholine. The increased discharge rate was evident and continued for several minutes. Concurrent stretching of the muscle accentuated the discharge rate. With addition of succinylcholine an increase in muscle tension of 40 mgm. was recorded by the strain gauge. Direct observation of the muscle with a binocular microscope showed a few extrafusal muscle fibers in sustained contraction. Presumptive evidence for activation of intrafusal and extrafusal muscle fibers by succinylcholine is thus present.

CHAPTER IV

DISCUSSION AND SUMMARY

DISCUSSION

1. Structure of muscle spindles

The general structure of muscle spindles in the lumbricals of the rat was similar to that already described in various muscles of the cat (3, 7, 17, 18, 23, 96), of the opossum (63) and of man (30). The intrafusal fibers differed in length and diameter but this difference was slight. The fibers could however be differentiated, according to the arrangement of their equatorial nuclei, into bags and chains.

The mean number of intrafusal fibers (3.5) present in each rat spindle was less than that found in the lumbrical muscles of the opossum (63) and in the fifth interosseous muscle of the cat's hindfoot (18), in both of which the mean spindle content was 8 to 9 intrafusal fibers. The mean fiber number for rat intrafusal fibers was less than the number of intrafusal fibers in the spindles of the soleus and gastrocnemius muscles of the cat which usually contained two nuclear bag fibers and four or five nuclear chain fibers per spindle (17, 18). The maximum number of intrafusal fibers observed by Boyd (18) in a single cat spindle was four nuclear bag and nine nuclear chain fibers. In human lumbrical and deep neck muscles, the spindles contained one to four nuclear bag and zero to ten nuclear chain fibers with a maximum of 14 (30). The maximum number observed in a rat spindle was two nuclear bag and three nuclear chain fibers.

No branching of intrafusal fibers was noted in the rat. Bridgman (21) did not find any branching of intrafusal fibers in the spindles of the extensor digitorum brevis muscle of the rat but did note branching and attachments to the

capsule of the majority of nuclear chain fibers in the cat and both nuclear chain and nuclear bag fibers in the gibbon. He felt that this increasing branching of intrafusal fibers, with attachments to the spindle capsule, from the rat to the gibbon was of some evolutionary significance and tied in with the hypothesis (22) that muscle spindles were capable of responding to changing pressures induced by compression of their periaxial space by contracting extrafusal fibers.

The diameters of nuclear bag fibers (16.1μ) and nuclear chain fibers (12.4μ) in the rat were similar to those of fibers in the interossei of the cat (18) in that the characteristic bimodal distribution was not present. The typical bimodal distribution was illustrated by the fibers in the extensor digitorum brevis of the cat (23), in which nuclear bag fibers had diameters of 11μ to 13μ and nuclear chain fibers 5μ to 7μ . In the lumbrical muscles of the opossum (63) the diameters of these fibers were respectively 15.4μ and 6.6μ which were similar to those for the cat's tenuissimus (16.9μ and 8.4μ) and for the cat's soleus (28.5μ and 11.6μ) (18). It appeared from Boyd's histograms (18), that intrafusal fiber diameters probably varied with the diameter of the extrafusal fibers of the muscle in which they lie, for the mean diameters of extrafusal fibers in the tenuissimus and soleus were 28μ and 54.1μ . Thus the small mean diameter of extrafusal fibers (30.7μ) in the lumbricals of the rat may have some bearing on the diameter of the intrafusal fibers.

The mean lengths of nuclear bag fibers (1.58mm) and nuclear chain fibers (1.20mm) were much smaller than that found in the interossei of the cat (18) and once again a bimodal distribution was not evident. In human lumbricals (30) the average length of the nuclear bag fiber was 5mm . and of the nuclear chain fiber 3.5mm . Mean lengths for nuclear bag and nuclear chain fibers in the soleus muscle of the

cat were 7.5mm. and 4mm. respectively (18). It was noteworthy that most nuclear chain fibers in the rat did not begin and end at the poles of the capsule as they did in human lumbricals (30) and in the larger leg muscles of the cat (18). The arrangement of nuclear chain fibers with respect to the poles of the capsule may, however, be related in some manner to the muscle in which they were situated, for the arrangement in the lumbrical muscles of the opossum (63), fifth interosseous muscle of the cat's hindfoot (18) and the lumbrical muscles of the rat are all similar.

The distribution of nuclei at the equatorial region of nuclear bag and nuclear chain fibers generally paralleled that in the cat (18) and the human (30). By definition, the nuclear chain fiber had a single axial row of nuclei and the nuclear bag fiber had a central bag region, containing more than one nucleus across its width, and flanking myotube regions made up of a single axial row of nuclei (2). Thus the specific distribution of nuclei within particular intrafusal fibers, coupled with their shape, frequently helped in the identification of nuclear bag and nuclear chain fibers since this could not always be done by noting differences in length and diameter of these fibers. The differences in nuclear shape, reflected by their length and width measurements, also held for the cat and allowed for identification of nuclear bag and nuclear chain fibers.

(2) Technical problems encountered in studying the spindle nerve supply.

In studying the motor innervation of spindle whole mounts various technical problems must be overcome. Deafferentation and deafferentation of the muscle under study was considered to be a necessary prerequisite in order to clarify the innervation especially in areas where motor and sensory fibers overlapped. The teasing process

must be performed with care so that the spindle could be removed, intact with its innervation, from the muscle. Adequate staining of spindle whole mounts is vitally important and has been a contentious issue. Both Boyd and Barker doubted whether the techniques used by the other were adequate to deal with all the problems at hand. This was exemplified by the remark of one on a certain statement of the other, that it was "the figment of a histological technique that is inadequate for demonstrating the finer details required" (3). Many staining methods have been advocated including gold chloride by Boyd (16), methylene blue also by Boyd (15), cholinesterase by Hess (46) and silver by Barker and Ip (9). Boyd (18) has used his gold chloride method to good advantage. Barker (1,4) has obtained good results using silver stained, as well as osmic acid stained, preparations. Jones (64) used methylene blue, gold, and silver stains in his study of opossum spindles. All of these staining methods, except the cholinesterase technique and Barker's silver technique, were attempted in this study. The best staining of structures within the spindle was obtained using a silver technique (99). The short comings of the capricious silver stains are well known and must be considered when interpreting whether the entire nerve supply has been adequately demonstrated.

(3) The motor innervation within the spindle

It is necessary to emphasize that this investigation was based entirely on observations made on the muscle nerve trunk and within 1mm. of individual spindles. While certain conclusions can be drawn from such a study, the degree of branching of nerve fibers before entering the field of observation in the muscle remains unknown.

Physiological evidence indicates that the muscle spindles of the cat have dynamic and static properties, which are each influenced by different gamma efferent nerve

fibers (60, 61). Morphological studies have clearly shown that most muscle spindles in most mammals possess two clearly distinct types of intrafusal muscle fiber. The important question, therefore, is whether there is any specificity in the innervation of these two types by the two types of gamma efferent fibers demonstrable physiologically. Several studies have been devoted to the elucidation of this problem but there is little agreement on whether nuclear bag and nuclear chain fibers are specifically innervated; nor is there agreement that the visible differences between their motor endings have in themselves a functional significance. Boyd (17, 18, 20) felt that, in the cat, nuclear bag and nuclear chain fibers each received gamma efferents which were distinguished by differences in diameter at the spindle and in the muscle nerves, and also, that the larger type ended specifically as end plates on nuclear bag fibers and that the smaller type ended specifically as diffuse networks on nuclear chain fibers. Barker and his colleagues, while admitting the presence of gamma efferents of different diameters, have always denied (1,3,5,10) that there is any specificity in their distribution to nuclear bag or nuclear chain fibers. Initially, they also denied (3,5) the existence of a diffuse nuclear chain ending claiming both nuclear bag and nuclear chain fibers always receive plate endings only. Subsequently, Barker (4, 10) has demonstrated diffuse "trial endings" in the cat, which seems to be equivalent to the diffuse network of Boyd, but he has shown that these endings occur indiscriminately on both bag and chain fibers. Moreover, he demonstrated that some nuclear chain fibers received plate endings as well as trail endings, and also that trail endings occur in the rabbit, whose spindles contain only nuclear bag fibers (8).

In the opossum, it was shown by Jones (64) that, while in two-thirds of spindles nuclear bag and nuclear chain fibers were supplied by axons that remained separate as far back as they could be traced, in at least one third some bag and chain endings arose from a single axon. In addition, it was shown that, while nuclear chain fibers commonly received diffuse spray-like endings and nuclear bag fibers had end plates, nevertheless, chain fibers often had end plates and bag fibers often had diffuse end plates and occasionally, sprays.

In the rat, nuclear bag and nuclear chain fibers were innervated in all cases by axons which remained separate as far back as they could be traced. Outside the spindle the two fusimotor axons could be distinguished by diameter, the larger axon usually innervating the nuclear bag fiber and the smaller axon the nuclear chain fiber. However, within the spindle this size distinction did not persist as both axons became of equal diameter. As described by Jones (64) for the opossum and by Barker (4) and Barker and Ip (10) for the cat, the type of motor nerve ending was not specific for a particular type of intrafusal fiber. Whereas, nuclear bag fibers usually received motor end plates and nuclear chain fibers received filamentous endings, various combinations of endings on these fibers occurred. It thus seemed likely that neither the morphology of the motor endings nor the diameters of gamma efferents close to the spindle were, alone, sufficient criteria for a functional separation of intrafusal fibers and their motor supply.

(4) The motor nerve endings

The morphology of the motor endings in the rat differed from that described by several authors in a variety of mammals. The nuclear bag end plates were smaller

than extrafusal end plates, which coincides with that found in man (30), but is the reverse of the situation in the opossum (64). There was frequently a considerable inequality in the number of nuclear bag end plates situated at different poles of the same spindle in contrast to the rabbit and cat where the numbers were approximately equal (2, 18).

Filamentous endings commonly found on the nuclear chain fibers probably were the equivalent of the "gamma-2 network" endings of Boyd (18), "trail endings" of Barker and Ip (10) and "diffuse muliterminal endings" of Hess (46) but they, in no way, resembled these endings. These filamentous endings were seen to lie in the juxta-equatorial and mid polar regions of the spindle and consisted of a single fine nerve strand, but lacked the arborizations of fine terminal branches as described by Boyd (18), Barker (4), Barker and Ip (10), Cooper and Daniel (30) and Cöers (25).

A possible explanation for these filamentous endings could possibly lie in Barker and Ip's (11) interpretation that the trail endings in the cat were plate endings undergoing replacement. They describe an early state, in this process, in which there is an outgrowth of an unmyelinated axon sprout from the terminal branch supplying the ageing end plate. On making contact with a muscle fiber the sprout initiates the formation of a new end plate. These sprouts have been observed by Hinsey (49), who called them "accessory fibers", and by Tiegs (97) who called them "ultraterminal fibers". There is evidence, in this study, that this process is occurring at extrafusal motor end plates. Should this be the case, then perhaps nuclear chain and nuclear bag fibers normally receive only one type of ending, the motor end

plate. In his study of rat muscle spindles, Landon (69) was able to find only the plate-type of motor ending.

As one to three motor endings were usually found on one pole of each intrafusal fiber, even though both poles were striated, then one must assume that muscle action potentials were propagated from the end plates at one pole perhaps over the entire length of the intrafusal muscle fiber. It would be unlikely that the pole lacking innervation would be passive.

(5) The motor nerves

There were generally fewer motor nerve fibers (2) entering the spindles of the rat than the number quoted by Jones (64) for the opossum (4 - 16) and by Boyd (18) for the cat (7 - 23), in all cases counted 1mm. from the spindle. The number of motor nerve endings was correspondingly fewer in the rat.

Boyd (17, 18) was of the opinion that nuclear bag end plates and nuclear chain networks in the cat were innervated by two different kinds of gamma efferents. These he called "gamma-1" ($2.5-4\mu$) and "gamma-2" ($1.5-2\mu$) on the basis of their axon diameters 1mm. from the spindle in gold chloride preparations. Within 1mm. of the rat spindle motor axon diameters of 2 sizes were present ($1.5-2\mu$ and $0.5-1\mu$) in silver preparations. Within the spindle the axon diameters became much the same. There was no specificity of termination on nuclear bag or nuclear chain fibers except that the larger axon usually innervated nuclear bag fibers.

Through degeneration experiments the composition of each of the lumbrical muscle nerve trunks was ascertained. The motor component (5 to 9 axons) made up about one third of the total fiber content (15 to 26 axons) of each nerve. There was

a ratio of approximately one motor axon, in the peripheral nerve, for each spindle (3 to 8 spindles in each muscle) in the individual muscles. Physiological evidence revealed the presence of 3 to 5 extrafusal motor units. The answer to the problem of supplying 2 motor axons to each spindle from the 2 to 4 axons remaining appeared to depend on whether single axons branched to innervate extra and intrafusal muscle fibers or whether fusimotor axons branched to supply intrafusal fibers in more than one spindle. Both methods of innervation were found in the rat. Branching of fusimotor axons has been shown to occur in the cat by Barker (4). The innervation of muscle spindles by branches of alpha efferent nerve fibers has been shown histologically to occur in the cat by Adal and Barker (1) and Boyd and Davey (19), in the rabbit by Barker (4) and has been suggested to occur in the rat tail muscles by Steg (93, 94). Physiological evidence for such innervation has been shown in the cat by Granit, Pompeiano and Waltman (42, 43), Bessou, Emonet - Denand and Laporte (12, 13), Rutledge and Haase (90) and Brown, Crowe and Matthews (24), in the rabbit by Diete-Spiff and Pascoe (33) and in the tail muscles of the rat by Kidd (65). Adal and Barker (1) were able to find only 5 examples of such branching in the cat. Six spindles were found in this study with this skeletofusimotor innervation. Because of technical problems the frequency of its occurrence could not accurately be determined histologically. If such fibers were numerous, their reflex activation would have the interesting consequence of producing muscle tonic contraction which would perhaps be accompanied by an increase of spindle primary ending responsiveness to phasic stretches.

(6) The sensory innervation

Each spindle had only one primary sensory ending which was composed of a series of spirals or rings and was similar to that described in the cat by Ruffini (87),

and Boyd (17, 18) and in the rabbit by Barker (2). The ring formations on the nuclear chain fibers were shorter ($147\ \mu$) than those on the nuclear bag fibers ($176\ \mu$) which is comparable with that found in the opossum (64). The overall length of the primary ending ($130 - 240\ \mu$) was similar to that given by Barker (2), Boyd (18) and Cooper and Daniel (30).

Secondary sensory endings were present in about one-half of all spindles, one being the commonest number. Barker (2) gave 0-2 as the range in the rabbit and Boyd (18) 0-5 in the cat. The secondary endings lay to one side of the primary ending and could mingle with it. The form of the secondary ending was similar to that described by Barker (2) in the rabbit and by Boyd (17, 18) in the cat. The overall length of the secondary ending was $100 - 260\ \mu$ which is shorter than that described by Barker (2) ($500\ \mu$, rabbit) and Boyd (17, 18) ($300 - 500\ \mu$, cat) but is about the same as that described by Cooper and Daniel (30) for man.

The mean diameter of group 1a and group 11 afferent fibers to rat muscle spindles, stained with osmic acid, when measured 1mm. from the spindle was $9.8\ \mu$ (range $8 - 11\ \mu$) and $7 - 8\ \mu$ (range $6 - 10\ \mu$) respectively. The diameters for comparable nerves in the rabbit given by Barker (2) were $8-12\ \mu$ and $6-9\ \mu$ and in the cat given by Boyd (17, 18) $12\ \mu$ and $6\ \mu$. It has been claimed by various authors (18, 64) that group 1a afferents do not supply more than one spindle, however one example of this has been found in the rat. Boyd (18) has claimed that secondary endings in adjacent spindles may be supplied by branches of the same group 11 afferent fiber and Swett and Eldred (95) gave physiological evidence for such an occurrence but this was never observed in the present study.

(7) Other nerve fibers in the spindle

The very small ($0.5\ \mu$) nonmyelinated nerve fibers occasionally seen in the

capsular region appeared to have no relationship to the intrafusal fibers. They were quite distinct from the other nerve fibers in the spindle and cannot be classified as "gamma-2 efferents" as Boyd (18) suggested was the case with similar fibers seen passing to the poles by Barker (2). Hines and Tower (47) and Hinsey (48) have observed similar fibers in the capsular region and felt that they might be sympathetic fibers ending only on blood vessels within the capsule and not on the intrafusal fibers. It was not possible to say if the small nerve fibers observed in this study innervated the capsule or merely blood vessels, which were also noted to be present in the capsular region. Cooper and Daniel (30) described fine fibers in relation to both the capsule and blood vessels, and Eldred, Schnitzlein and Buchwald (38) and Hunt (54) have shown that stimulation of the sympathetic trunk in the cat would affect the afferent discharge from a muscle as recorded from dorsal root filaments.

(8) Evidence for two morphological intrafusal muscle fiber types.

The evidence produced thus far in this study favors the separation of nuclear bag and nuclear chain fibers into two distinct morphological types. There is evidence for independent motor innervation, with apparently two types of motor ending, of the nuclear bag and nuclear chain fibers. Landon (69), who has carried out an examination of spindles from the rat lumbrical muscles with the electron microscope, states that the nuclear bag fibers lack a "M" band, whereas the nuclear chain fibers have a prominent "M" band. A difference in the "M" band of the nuclear bag and nuclear chain fibers of the cat has been documented by Hubbard and Hess (51). Landon (69) feels, from the differences which he sees in the fine structure of the two types of intrafusal fiber, that the nuclear chain fiber most closely resembles the frog muscle "twitch" fiber, and the nuclear bag fiber resembles the frog "slow" fiber. Only motor plate

endings on the extracapsular regions of the nuclear bag fibers were identified by Landon (69). He noted that this motor ending, which lacked any organized subneural apparatus, was very similar to the endings located on the slow muscle fibers of the garter snake and on the slow muscle fibers of the frog. He concluded that "it may be that this similarity has some significance in regard to the modes of physiological activity of which the intrafusal muscle fibers are capable" (69).

Smith (92) concludes, from observations made after direct stimulation of spindles from rat lumbrical muscles, that the rat spindle contains two types of intrafusal muscle fiber having different contractile and mechanical properties. He noted that the nuclear bag fiber behaved as though it were a slow fiber and the nuclear chain as though it were a "twitch" or fast fiber. With addition of succinylcholine he observed a strong prolonged contracture in the slow intrafusal (nuclear bag) muscle fiber.

Succinylcholine is believed to excite spindle endings by causing the intrafusal muscle fibers to contract along their entire length (39). This action, which simulates the afferent response recorded from the spindle after stimulation of dynamic fusimotor fibers, is most pronounced on the slow nuclear bag fibers (85).

In this study an accelerated afferent discharge was recorded from the spindle on addition of succinylcholine. In addition, an increase in muscle tension of 40mgm. was recorded and scattered extrafusal muscle fibers were noted, by direct observation, to be in sustained contraction. Histologically, in all instances of skeletofusimotor innervation the nuclear bag fiber was thus innervated. Thus it appears that beta innervation, as it occurs in the rat, is composed of slow extra and intrafusal motor units. In the cat, Bessou, Emonet-Denand and Laporte (13) have concluded "that on the basis

of their effects on primary endings, the slow alpha fibers innervating both extra and intrafusal muscle fibers can be considered as dynamic fibers". They suggest that these slow alpha fibers innervate nuclear bag fibers but they could not exclude innervation also of the nuclear chain fibers.

Using physiological techniques, Smith (92) was able to predict, on the basis of different properties of the intrafusal fibers, that the intrafusal muscle fibers would be separately innervated. From a histological stand point there is ample evidence to support a hypothesis advocating two distinct intrafusal fiber morphological types in the rat. Such a scheme would agree with Boyd's (17, 18) morphological concept of the cat muscle spindle and the apparent functional independence of the two types of muscle fibers as proposed by Jansen and Matthews (60, 61).

SUMMARY

1. The morphology and innervation of muscle spindles in the plantar lumbrical muscles of the rat's hindpaw was determined by examining normal, as well as deafferented material. Intrafusal muscle fiber morphology was ascertained by reconstruction of spindles from serial cross sections of lumbrical muscles. Spindle innervation was determined through examination of teased whole spindles stained with silver.

Provisional conclusions, from this study, should be made keeping in mind that most of the interpretations are based on silver-stained specimens. The problem exists as to whether adequate staining of all the terminal axons in the spindle is occurring. In spite of studying rat muscle spindles which are simple, in that fewer muscle and nerve fibers are present, it is not possible to be absolutely sure that the motor innervation in the spindle is demonstrated in its entirety.

2. The nerve fiber content of each of the lumbrical muscle nerves was determined in normal, deafferented and deafferented specimens.
3. Each spindle contained three to five intrafusal fibers. Most of the spindles contained two nuclear bag and two nuclear chain fibers neither of which were seen to branch.
4. The length and diameter measurements of the nuclear chain fibers were smaller than the nuclear bag fibers; however some overlap in measurement occurred making it difficult, at times, to identify the type of fiber.
5. Equatorial nuclear aggregations and variations in nuclear shape were helpful in identification of intrafusal fiber type provided that the particular muscle fiber could be traced from the nucleated region to the spindle poles.

6. The motor innervation of the individual intrafusal fibers was separate with a larger motor axon usually innervating a nuclear bag fiber through motor end plates and a smaller motor axon usually innervating a nuclear chain fiber through filamentous endings.

7. One third of the total nerve fiber content of the lumbrical muscle nerves was of motor origin. It was noted that there were two motor axons entering each spindle, but at the nerve trunk level a ratio of one motor axon for each spindle in the muscle was found. In addition, by stimulating the muscle nerve, three to five extrafusal motor units were recorded. To explain the discrepancy between the number of motor axons available and that required to make up three to five motor units as well as innervate four to eight muscle spindles, then motor axon branching must take place. Such branching was present in the form of skeletofusimotor, or beta, innervation and also fusimotor axon branching for innervation of intrafusal fibers in more than one spindle.

8. Conduction velocity characteristics of the nerve trunk to lumbrical muscle 5 revealed that the most rapidly conducting nerve fibers were sensory, thus confirming the nerve fiber diameter histograms which were plotted for each nerve. A conduction velocity-temperature dependence of $1\text{M/sec}/^{\circ}\text{C}$ occurred in the larger sensory and motor axons. Smaller axons did not have the same degree of temperature dependence.

9. Sensory nerve endings in the spindle were similar to those described in other mammals.

10. Dynamic and static afferent responses to muscle stretch were recorded.

11. The sensitivity of some extra and intrafusal muscle fibers to the addition of succinylcholine was recorded. Increased afferent discharge was noted as well as

an increase in muscle tension of 40mgm. Thus from this information, and that extrapolated from the literature, it is evident that there are extra and intrafusal slow and fast motor units. The nuclear chain fiber is of the "twitch" or fast variety, whereas the nuclear bag fiber is of the slow type. Where beta innervation occurs, the stem motor axon innervates the slow intrafusal, or nuclear bag, motor unit and a slow extrafusal motor unit.

12. Adequate histological and physiological evidence is present for the separation of the nuclear bag fibers and the nuclear chain fibers into independently functional units within the spindle.

1. Smith, J. (1995). *Business and Society: A Practical Approach*. London: Prentice Hall.
2. Miller, D. (1998). *The Business Case for Sustainability*. London: Prentice Hall.
3. Jones, M. (2000). *Business Ethics: A Practical Approach*. London: Prentice Hall.
4. Miller, D. (2002). *The Business Case for Sustainability*. London: Prentice Hall.
5. Miller, D. (2005). *The Business Case for Sustainability*. London: Prentice Hall.
6. Miller, D. (2008). *The Business Case for Sustainability*. London: Prentice Hall.
7. Miller, D. (2011). *The Business Case for Sustainability*. London: Prentice Hall.
8. Miller, D. (2014). *The Business Case for Sustainability*. London: Prentice Hall.
9. Miller, D. (2017). *The Business Case for Sustainability*. London: Prentice Hall.
10. Miller, D. (2020). *The Business Case for Sustainability*. London: Prentice Hall.

BIBLIOGRAPHY

1. Smith, J. (1995). *Business and Society: A Practical Approach*. London: Prentice Hall.
2. Miller, D. (1998). *The Business Case for Sustainability*. London: Prentice Hall.
3. Jones, M. (2000). *Business Ethics: A Practical Approach*. London: Prentice Hall.
4. Miller, D. (2002). *The Business Case for Sustainability*. London: Prentice Hall.
5. Miller, D. (2005). *The Business Case for Sustainability*. London: Prentice Hall.
6. Miller, D. (2008). *The Business Case for Sustainability*. London: Prentice Hall.
7. Miller, D. (2011). *The Business Case for Sustainability*. London: Prentice Hall.
8. Miller, D. (2014). *The Business Case for Sustainability*. London: Prentice Hall.
9. Miller, D. (2017). *The Business Case for Sustainability*. London: Prentice Hall.
10. Miller, D. (2020). *The Business Case for Sustainability*. London: Prentice Hall.

- (1) Adal, M.N., and D. Barker. Intrafusar branching of fusimotor fibers. *J. Physiol. (London)* 177: 288-299, 1965.
- (2) Barker, D. The innervation of the muscle spindle. *Quart. J. Microscop. Sci.* 89: 143 - 186, 1948.
- (3) Barker, D. The structure and distribution of muscle receptors. In: Symposium on Muscle Receptors, edited by D. Barker, Hong Kong: Hong Kong Univ. Press, 1962, pp. 227-240.
- (4) Barker, D. The motor innervation of the mammalian muscle spindle. In: Muscular Afferents and Motor Control, edited by R. Granit. Almquist and Wiksell, Stockholm, 1966, pp 51 - 58.
- (5) Barker, D., and M. Cope. The innervation of individual intrafusar muscle fibers. In: Symposium on Muscle Receptors, edited by D. Barker. Hong Kong: Hong Kong Univ. Press, 1962, pp 263 - 269.
- (6) Barker, D., M. Cope, and M.C. Ip. The innervation of intrafusar muscle fibers in the cat. *Proc. XXII Int. Congr. Physiol. Sci. Abstr.* 916. 1962.
- (7) Barker, D., and J.L. Gidumal. The morphology of intrafusar muscle fibers in the cat. *J. Physiol. (London)* 157: 513 - 528, 1961.
- (8) Barker, D., and J.P. Hunt. Mammalian intrafusar muscle fibers. *Nature (London)* 203: 1193, 1964.
- (9) Barker, D. and M.C. Ip. A silver method for demonstrating the innervation of mammalian muscle in teased preparations. *J. Physiol. (London)* 169: 73 - 74P, 1963.
- (10) Barker, D. and M.C. Ip. The motor innervation of cat and rabbit muscle spindles. *J. Physiol. (London)* 177: 27 - 28 P, 1965.
- (11) Barker, D. and M.C. Ip. The probable existence of a process of motor end-plate replacement. *J. Physiol. (London)* 176: 11 - 12P, 1965.
- (12) Bessou, P., F. Emonet-Denand, and Y. Laporte. Occurrence of intrafusar muscle fiber innervation by branches of slow alpha motor fibers in the cat. *Nature* 198: 594 - 595, 1963.
- (13) Bessou, P., F. Emonet-Denand, and Y. Laporte. Increase of primary endings dynamic sensitivity by slow alpha fibers innervating muscle spindles. *Life Sci.* 2: 948 - 952, 1963.
- (14) Bessou, P., and Y. Laporte. Responses from primary and secondary endings of the

same neuromuscular spindle of the tenuissimus muscle of the cat. In: Symposium on Muscle Receptors, edited by D. Barker. Hong Kong: Hong Kong Univ. Press, 1962, pp 105 - 119.

- (15) Boyd, I. A. A methylene-blue technique for staining muscle spindles. *J. Physiol. (London)* 144: 10 - 11P, 1958.
- (16) Boyd, I. A. Uniform staining of nerve endings in skeletal muscle with gold chloride. *Stain Techn.* 37: 225 - 230, 1962.
- (17) Boyd, I. A. The nuclear bag fiber and nuclear chain fiber systems in the muscle spindles of the cat. In: Symposium on Muscle Receptors, edited by D. Barker. Hong Kong: Hong Kong Univ. Press, 1962, pp 185 - 190.
- (18) Boyd, I. A. The structure and innervation of the nuclear bag muscle fiber system and the nuclear chain muscle fiber system in mammalian muscle spindles. *Phil. Trans. Roy. Soc. London, Ser. B.* 245: 81 - 136, 1962.
- (19) Boyd, I. A. and M. R. Davey. Beta efferent fibers in nerves to mammalian skeletal muscle. *J. Physiol. (London)* 149: 28 - 29P, 1959.
- (20) Boyd, I. A. and M. R. Davey. The groups of origin in the nerves to skeletal muscle of the gamma and gamma-2 fusimotor fibers present close to, and within, mammalian muscle spindles. In: Symposium on Muscle Receptors, edited by D. Barker. Hong Kong: Hong Kong Univ. Press, 1962, pp 191 - 198.
- (21) Bridgman, C. F. Variations in insertions of intrafusal muscle fibers in spindles: possible significance. *Anat. Rec.* 157: 219, 1967.
- (22) Bridgman, C. F. and E. Eldred. Hypothesis for a pressure-sensitive mechanism in muscle spindles. *Science* 143: 481 - 482, 1964.
- (23) Bridgman, C. F., E. Eldred and B. Eldred. Distribution and structure of muscle spindles in the extensor digitorum brevis of the cat. *Anat. Rec.* 143: 219 - 227, 1962.
- (24) Brown, M. C., A. Crowe, and P. B. C. Matthews. Observations on the fusimotor fibers of the tibialis posterior muscle of the cat. *J. Physiol. (London)* 177: 140 - 159, 1965.
- (25) Coërs, C. Histochemical identification of motor nerve endings in muscle spindles. In: Symposium on Muscle Receptors, edited by D. Barker. Hong Kong: Hong Kong Univ. Press, 1962, pp 221 - 226.

- (26) Cöers, C. and J. Durand. Donnees morphologiques nouvelles sur l'innervation des fuseaux neuromusculaires. Arch. Biol. 67: 685 - 715, 1956.
- (27) Cole, W.V. Motor endings in the striated muscle of vertebrates. J. Comp. Neurol 108: 445 - 464, 1957.
- (28) Cooper, S. Muscle spindles and other muscle receptors. In: The Structure and Function of Muscle, edited by G.H. Bourne. New York: Academic Press, 1960, 1: 381 - 420.
- (29) Cooper, S., and P.M. Daniel. Muscle spindles in human extrinsic eye muscles. Brain 72: 1 - 24, 1949.
- (30) Cooper, S., and P.M. Daniel. Muscle spindles in man; their morphology in the lumbricals and the deep muscles of the neck. Brain 86: 563 - 586, 1963.
- (31) Cuajunco, F. Embryology of the neuromuscular spindle. Contr. Embryol. Carnegie Inst. 99, vol. 19: 45-72, 1927.
- (32) Cuajunco, F. Development of the neuromuscular spindle in human fetuses. Contr. Embryol. Carnegie Inst. 173, vol. 28: 95 - 128, 1940.
- (33) Diete - Spiff, K., and J.E. Pascoe. The spindle motor nerves to the gastrocnemius muscle of the rabbit. J. Physiol.(London) 149: 120 - 134, 1959.
- (34) Eccles, J.C., and C.S. Sherrington. Numbers and contraction-values of individual motor units examined in some muscles of the limb. Proc. Roy. Soc. London, B106: 326 - 357, 1930.
- (35) Eccles, R.M., and A. Lundberg. Integrative pattern of Ia synaptic actions on motoneurons of hip and knee muscles. J. Physiol. (London) 144: 271 - 298, 1958.
- (36) Eccles, R.M., and A. Lundberg. Synaptic actions in motoneurons by afferents which may evoke the flexion reflex. Arch. Ital. Biol. 97: 199 - 221, 1959.
- (37) Eldred, E., R. Granit, and P.A. Merton. Supraspinal control of the muscle spindle and its significance. J. Physiol. (London) 122: 498 - 523, 1953.
- (38) Eldred, E., H.N. Schnitzlein, and J. Buchwald. Response of muscle spindles to stimulation of the sympathetic trunk. Expl. Neurol. 2: 13 - 25, 1960.
- (39) Fehr, H.U. Activation by Suxamethonium of primary and secondary endings of the same de-efferented muscle spindle during static stretch. J. Physiol. (London) 178: 98 - 110, 1965.

- (40) Fullerton, P.M. and R.W. Gilliatt. Changes in nerve conduction in caged guinea-pigs. *J. Physiol. (London)* 178: 47 - 48P, 1965.
- (41) Granit, R., and S. Homma. The discharge to maintained stretch of spindles in slow and fast muscles of rabbit. *Acta Physiol. Scand.* 46: 165 - 173, 1959.
- (42) Granit, R., O. Pompeiano, and B. Waltman. Fast Supraspinal control of mammalian muscle spindles: extra and intrafusal co-activation. *J. Physiol. (London)* 147: 385 - 398, 1959.
- (43) Granit, R., O. Pompeiano, and B. Waltman. The early discharge of mammalian muscle spindles at onset of contraction. *J. Physiol. (London)* 147: 399 - 418, 1959.
- (44) Greene, E.C. Anatomy of the rat. In: Transactions of the American Philosophical Society. Hafner Publishing Co., New York, 1963. 27: 115 - 175.
- (45) Harvey, R.J., and P.B.C. Matthews. The response of de-efferented muscle spindle endings in the cat's soleus to slow extension of the muscle. *J. Physiol. (London)* 157: 370 - 392, 1961.
- (46) Hess, A. Two kinds of motor nerve endings on mammalian intrafusal muscle fibers revealed by the cholinesterase technique. *Anat. Rec.* 139: 173 - 184, 1961.
- (47) Hines, M., and S.S. Tower. Studies on the innervation of skeletal muscles. 2: of muscle spindles in certain muscles of the kitten. *Bull. Johns Hopkins Hosp.* 42: 264 - 307, 1928.
- (48) Hinsey, J.C. Some observations on the innervation of skeletal muscle of the cat. *J. Comp. Neurol.* 44: 87 - 195, 1927.
- (49) Hinsey, J.C. The innervation of skeletal muscle. *Physiol. Rev.* 14: 514 - 585, 1934.
- (50) Hofmann, W.W. Observations on peripheral servo mechanisms in Parkinsonian reigidity. *J. Neurol. Neurosurg. Psychiat.* 25: 203 - 207, 1962.
- (51) Hubbard, S.J. and A. Hess. The fine structure of the primary sensory zone of cat muscle spindles. *Anat. Rec.* 157: 262 - 263, 1967.
- (52) Huber, G.H., and L.M.A. DeWitt. A contribution on the motor nerve-ending and on the nerve-endings in the muscle spindles. *J. Comp. Neurol.* 7: 169 - 230, 1897.
- (53) Hunt, C.C. Relation of function to diameter in afferent fibers of muscle nerves. *J. Gen. Physiol.* 38: 117 - 131, 1954.

- (54) Hunt, C.C. The effect of sympathetic stimulation on mammalian muscle spindles. J. Physiol. (London) 151: 332 - 341, 1960.
- (55) Hunt, C.C., and S.W. Kuffler. Further study of efferent small nerve fibers to mammalian muscle spindles. Multiple spindle innervation and activity during contraction. J. Physiol. (London) 113: 283 - 297, 1951.
- (56) Hunt, C.C., and S.W. Kuffler. Stretch receptor discharges during muscle contraction. J. Physiol. (London) 113: 298 - 315, 1951.
- (57) Hunt, C.C., and A.S. Paintal. Spinal reflex regulation of fusimotor neurones. J. Physiol. (London) 143: 195 - 212, 1958.
- (58) Jansen, J.K.S. Spasticity - Functional aspects. Acta Neurol. Scand. 38, Suppl. 3: 41 - 51, 1962.
- (59) Jansen, J.K.S., and P.B.C. Matthews. The dynamic responses to slow stretch of muscle spindles in the decerebrate cat. J. Physiol. (London) 159: 20 - 22P, 1961.
- (60) Jansen, J.K.S., and P.B.C. Matthews. The central control of the dynamic response of muscle spindle receptors. J. Physiol. (London) 161: 357 - 378, 1962.
- (61) Jansen, J.K.S., and P.B.C. Matthews. The effects of fusimotor activity on the static responsiveness of primary and secondary endings of muscle spindles in the decerebrate cat. Acta Physiol. Scand. 55: 376-386, 1962.
- (62) Jansen, J.K.S., and T. Rudjord. The silent period during twitch contraction of the soleus of the decerebrate cat. Acta Physiol. Scand. 59, Suppl. 213: 69 - 70, 1963.
- (63) Jones, E.G. Structure and distribution of muscle spindles in the forepaw lumbricals of the phalanger, Trichosurus vulpecula. Anat. Rec. 155: 287 - 304, 1966.
- (64) Jones, E.G. The innervation of muscle spindles in the Australian opossum, Trichosurus vulpecula, with special reference to the motor nerve endings. J. Anat. 100: 733 - 759, 1966.
- (65) Kidd, G.L. An analysis of beta axon excitation of the muscle spindle in the rat. In: Control and Innervation of Skeletal Muscle, edited by B.L. Andrew. Thomson & Co. Ltd: Dundee, 1966, pp 83 - 94.
- (66) Kuffler, S.W., and C.C. Hunt. The mammalian small nerve fibers; a system for efferent nervous regulation of muscle spindle discharge. Res. Publ. Assoc. Res. Nervous Mental Disease 30: 24 - 47, 1952.

- (67) Kuffler, S.W., C.C. Hunt, and J.P. Quilliam. Function of medullated small nerve fibers in mammalian ventral roots: efferent muscle spindle innervation. *J. Neurophysiol.* 14: 29 - 54, 1951.
- (68) Landau, W.M., R.A. Weaver, and T.F. Hornbein. Fusimotor nerve function in man. *Arch. Neurol.* 3: 10 - 23, 1960.
- (69) Landon, D.N. Electron microscopy of muscle spindles. In: Control and Innervation of Skeletal Muscle, edited by B.L. Andrews. Thomson & Co. Ltd: Dundee, 1966, pp 96 - 110.
- (70) Langley, J.N. The nerve fiber constitution of peripheral nerves and of nerve roots. *J. Physiol. (London)* 56: 382 - 396, 1922.
- (71) Leksell, L. The action potential and excitatory effects of the small ventral root fibers to skeletal muscle. *Acta Physiol. Scand.* 10, Suppl. 31: 1 - 84, 1945.
- (72) Liley, A.W. An investigation of spontaneous activity at the neuromuscular junction of the rat. *J. Physiol.* 132: 650 - 666, 1956.
- (73) Matthews, B.H.C. Nerve endings in mammalian muscle. *J. Physiol. (London)* 78: 1 - 53, 1933.
- (74) Matthews, P.B.C. The differentiation of two types of fusimotor fiber by their effects on the dynamic response of muscle spindle primary endings. *Quart. J. Exptl. Physiol.* 47: 324 - 333, 1962.
- (75) Matthews, P.B.C. The response of de-efferented muscle spindle receptors to stretching at different velocities. *J. Physiol. (London)* 168: 660 - 678, 1963.
- (76) Matthews, P.B.C. Muscle spindles and their motor control. *Physiol. Rev.* 44: 219 - 288, 1964.
- (77) Merrillees, N.C.R. The fine structure of muscle spindles in the lumbrical muscles of the rat. *J. Biophys. Biochem. Cytol.* 7: 725 - 742, 1960.
- (78) Merrillees, N.C.R., Sunderland, and W. Hayhow. Neuromuscular spindles in the extraocular muscles in man. *Anat. Rec.* 108: 23-30, 1950.
- (79) Merton, P.A. Significance of the silent period of muscles. *Nature* 166: 733-734, 1950.
- (80) Merton, P.A. The silent period in a muscle of the human hand. *J. Physiol. (London)* 114: 183 - 198, 1951

- (81) Merton, P. A. Speculations on the servo-control of movement. In: The Spinal Cord, edited by G. E. W. Wolstenholme. Churchill: London, 1953, pp 247 - 255.
- (82) Merton, P. A. Slowly conducting muscle spindle afferents. *Acta Physiol. Scand.* 29: 87 - 88, 1953.
- (83) Page, S. G., and H. E. Huxley. Filament lengths in striated muscle. *J. Cell Biol.* 19: 369 - 390, 1963.
- (84) Partridge, L. D., and G. H. Glaser. Adaptation in regulation of movement and posture. A study of stretch responses in spastic animals. *J. Neurophysiol.* 23: 257 - 268, 1960.
- (85) Rack, P. M. H., and D. R. Westbury. The effects of Suxamethonium and acetylcholine on the behavior of cat muscle spindles during dynamic stretching, and during fusimotor stimulation. *J. Physiol. (London)* 186: 698 - 713, 1966.
- (86) Ruffini, A. Observations on sensory nerve - endings in voluntary muscles. *Brain* 20: 368 - 374, 1897.
- (87) Ruffini, A. On the minute anatomy of the neuromuscular spindles of the cat, and on their physiological significance. *J. Physiol. (London)* 23: 190 - 208, 1898.
- (88) Rushworth, G. Spasticity and rigidity: an experimental study and review. *J. Neurol. Neurosurg. Psychiat.* 23: 99 - 118, 1960.
- (89) Rushworth, G. The gamma system in Parkinsonism. *Int. J. Neurol.* 2: 34-50, 1961.
- (90) Rutledge, L. T., and J. Haase. Flexor muscle spindles and reflex firing of early discharging units. *J. Neurophysiol.* 24: 182 - 192, 1961.
- (91) Sherrington, C. S. On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibers in the ventral spinal nerve-root. *J. Physiol. (London)* 17: 211 - 258, 1894.
- (92) Smith, R. S. Properties of intrafusal muscle fibers. In: Muscular Afferents and Motor Control, edited by R. Granit. Almquist and Wiksell: Stockholm, 1966, pp 69-78.
- (93) Steg, G. The function of muscle spindles in spasticity and rigidity. *Acta Neurol. Scand.* 38, Suppl. 3: 53-59, 1962.

- (94) Steg. G. Efferent muscle innervation and rigidity. *Acta Physiol. Scand.* 61, Suppl. 225: 1-53, 1964.
- (95) Swett, J.E., and E. Eldred. Relation between spinal level and peripheral location of afferents in calf muscles of the cat. *Am. J. Physiol.* 196: 819 - 823, 1959.
- (96) Swett, J.E., and E. Eldred. Comparisons in structure of stretch receptors in medial gastrocnemius and soleus muscles of the cat. *Anat. Rec.*, 137: 461-473, 1960.
- (97) Tiegs, O.W. Innervation of voluntary muscle. *Physiol. Rev.* 33: 90 - 144, 1953.
- (98) Williams, P.L., and C.P. Wendell-Smith. The use of fixed and stained sections in quantitative studies of peripheral nerve. *Quart. J. Micr. Sci.* 101: 43 - 54, 1960.
- (99) Winkelmann, R.K., and R.W. Schmit. A simple silver method for nerve axoplasm. *Proc. Mayo Clin.* 32: 217 - 222, 1957.
- (100) Zacks, S.I. The Motor Endplate. Saunders: Philadelphia, 1964, pp 258 - 259.

TABLE 1. Summary of the results of the analysis of variance for the different parameters measured.

TABLE 2. Summary of the results of the analysis of variance for the different parameters measured.

Parameter	df	SS	MS	F	P	Q
Mean weight (g)	1, 10	1.2	1.2	1.2	0.3	0.1
Mean length (mm)	1, 10	0.5	0.5	0.5	0.5	0.1
Mean girth (mm)	1, 10	0.1	0.1	0.1	0.9	0.1
Mean 1 week mean of 10 days (mm)	1, 10	0.1	0.1	0.1	0.9	0.1
Mean 1 week mean of 10 days (mm)	1, 10	0.1	0.1	0.1	0.9	0.1
Mean 1 week mean of 10 days (mm)	1, 10	0.1	0.1	0.1	0.9	0.1

TABLES AND FIGURES

Table 1

Muscle weights, spindle content and muscle nerve composition of six plantar lumbrical muscles.

Lumbrical Muscle	MC	LC	2	3	4	5
Muscle Weight (wet) (Mgm)	3	4	4.5	4	5	6.5
Number of Spindles	1-3	1-3	3-5	4-6	3-4	4-8
Total Nerve Fibers In Muscle Nerve	17	21	15	18	22	26
Total Sensory Fibers In Muscle Nerve	12	10	23	19	20	18
Total Motor Fibers In Muscle Nerve	5	5	5	6	7	9

Fig. 1. Sizes of plantar lumbrical muscles, their spindle receptor content, and the manner of origin from the tendon flexor hallucis longus. M. C., medial central lumbrical. L. C., lateral central lumbrical. 2, 3, 4, 5, lumbricals inserting on the proximal phalanges of digits 2, 3, 4, 5, respectively. The number and length of the muscle spindles are taken from observations of specimens stained with gold chloride. Spindle capsules are indicated by dots on the full lines. The lumbricals M. C. and L. C. are drawn as though reflected back.

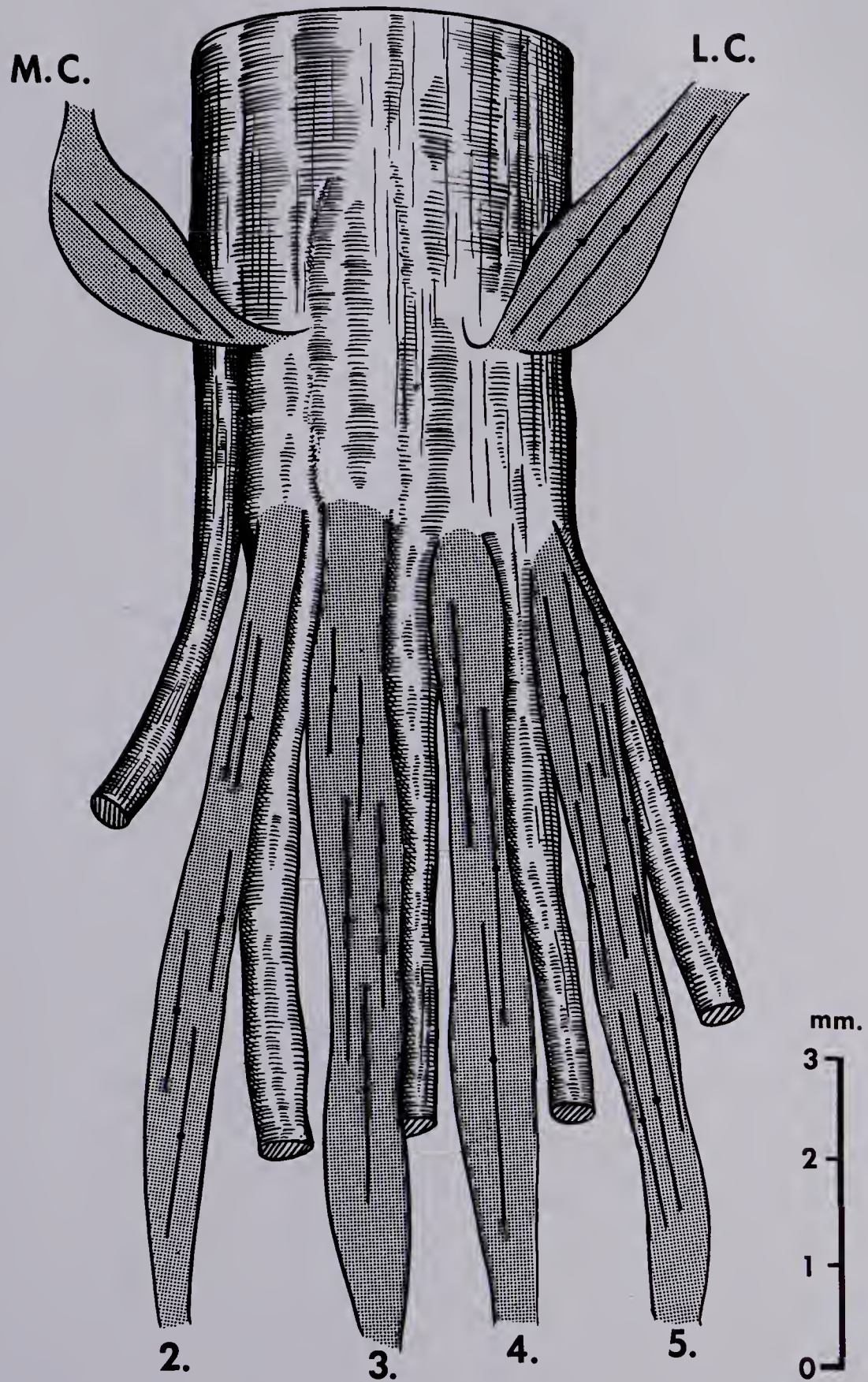


FIG. I

Fig. 2. Gross innervation of the hindpaw plantar lumbrical muscles as determined by dissection of the appropriate nerves and muscles and electrical stimulation of the spinal roots. The medial plantar nerve (MPN) branches to innervate the following lumbrical muscles: lateral central (LC), medial central (MC), lumbrical to 2nd digit (2), 3rd digit (3) and 4th digit (4). The lateral plantar nerve (LPN) innervates the lumbrical muscle to the 5th digit (5) and occasionally innervates the 4th lumbrical with the MPN, as indicated by the dotted line. The dashed lines across the various nerves indicates the level of section from which specimens were taken for cross section.

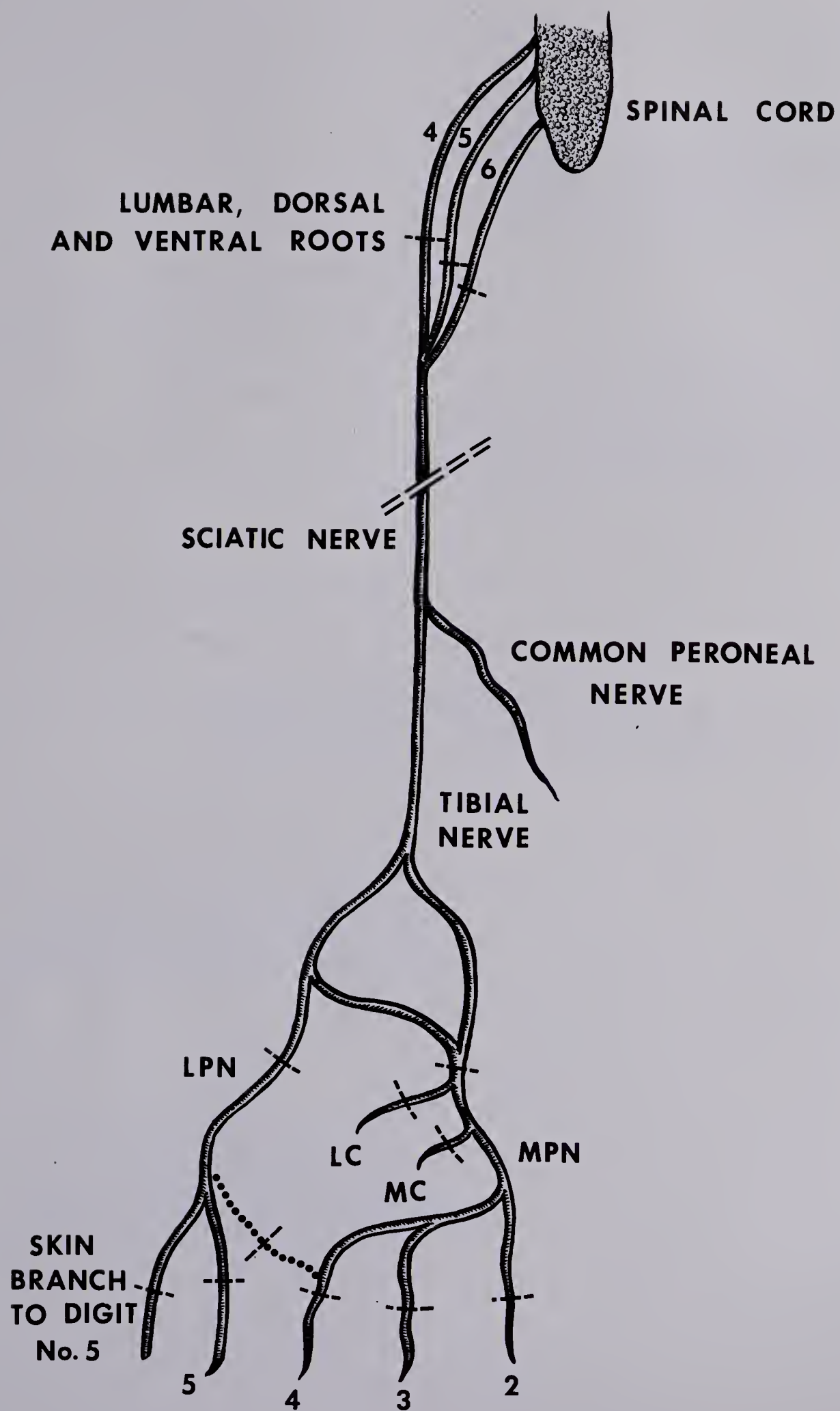


FIG. 2

Fig. 3. Five examples of muscle spindles reconstructed from serial cross sections of lumbrical muscles fixed relaxed glutaraldehyde, embedded in paraffin and stained with hematoxylin and eosin.

The lines (a) to (f) on spindle no. I represent the levels of section corresponding to the spindle cross sections (a) to (f) shown in Fig. 4.

Spindle no. III does not contain a nuclear bag muscle fiber. Nuclear chain fibers contain the characteristic single, axial row of elongated nuclei in contrast to the nuclear bag fibers which contain 2 - 3 oval nuclei abreast in the central region. The nuclei are not drawn to scale.

The nuclear chain fibers are seen to vary from being almost entirely intracapsular (spindle no. IV) to extending the entire length of the spindle (spindle no. V). The characteristic thinning of the nuclear chain equatorial region and clubbing of the polar region is seen.

Note the changes in the scale representing the length of the spindle. In spindles I and II width is 10 x length, in III and IV width is 5 x length and in V width is 20 x length.

Scales are not corrected for shrinkage.

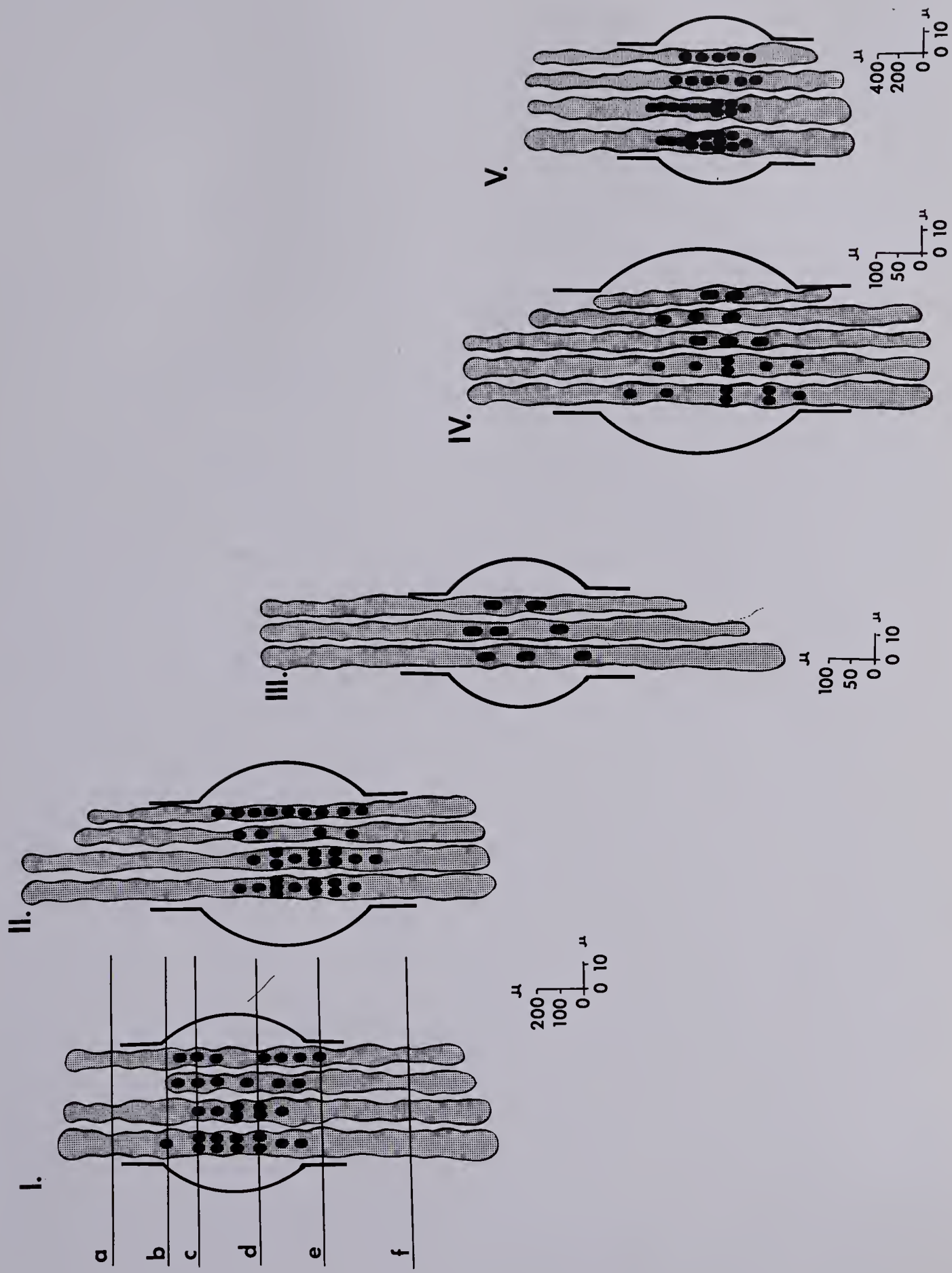


FIG. 3

Fig. 4. Cross sections of muscle spindles.

A. shows the polar ends of two spindles (sp), the lower spindle continues through sections B to F. Sections (A) through (F) correspond to the levels (a) through (f) of Fig. 3. Section (C) is labelled to show the spindle capsule (c), a nerve fiber (n), two nuclear chain muscle fibers (nc) and two nuclear bag muscle fibers (nb).

The sections were cut from glutaraldehyde fixed, paraffin embedded material sectioned at 7 μ and stained with hematoxylin and eosin.

Scale bar — 20 μ .

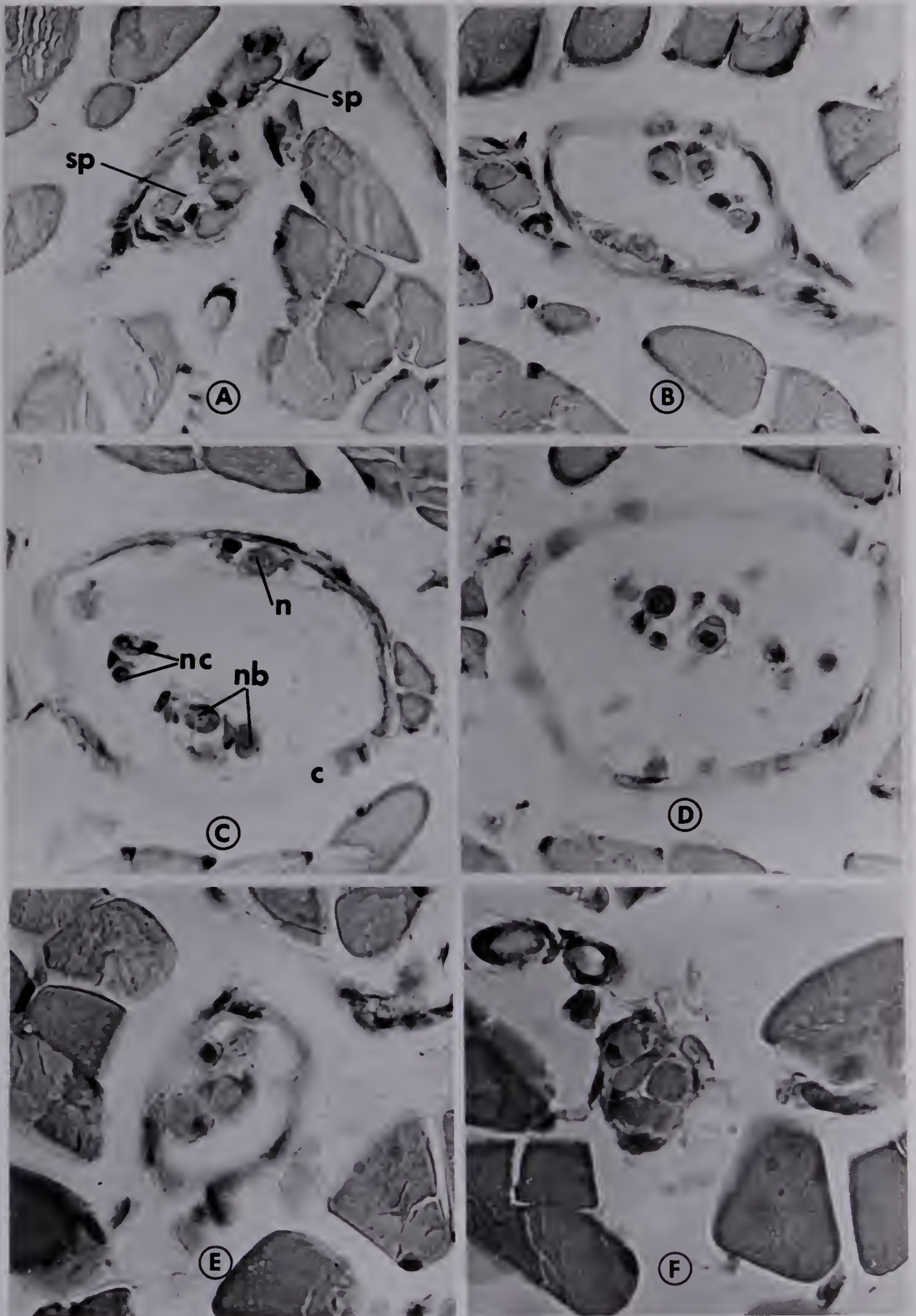


FIG. 4

Fig. 5. Low and high power photomicrographs of muscle cross sections to show:

- A. The entire muscle fiber content of lumbrical muscle 3. A spindle sectioned through its equatorial region is circled.
- B. An oil - immersion view of a muscle spindle. Three intrafusal muscle fibers and a nerve terminal (n) are contained in the axial capsule (a). The nuclei in the two left - hand intrafusal fibers are indicated (nuc.) Part of the spindle capsule (c) can be seen at the right - hand side of the photograph.

7 μ paraffin sections stained with hematoxylin and eosin.

Scale bar - A, 250 μ ; B, 10 μ .

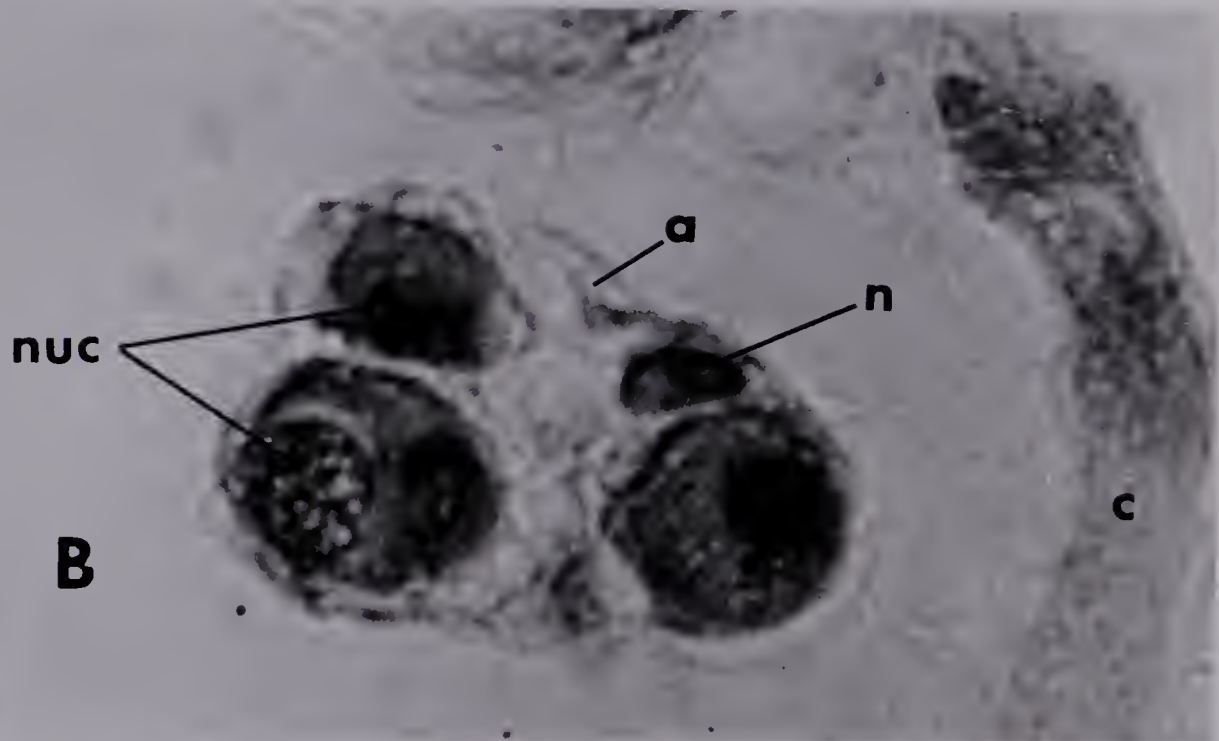


FIG. 5

Fig. 6. Comparison of extrafusar muscle fiber diameter distributions of a lumbrical muscle fixed relaxed, embedded in paraffin and stained with hematoxylin and eosin (oblique hatching to the left) with a comparable lumbrical muscle fixed in situ, stained with osmic acid and embedded in araldite (oblique hatching to the right).

Mean muscle diameter of paraffin embedded material 21.8μ (± 1.4); $n = 244$. Mean diameter of araldite embedded material 30.7μ (± 1.6); $n = 308$. n = number of fibers measured.

Comparison of the two means indicates a shrinkage of 29% occurring in the paraffin embedded material.

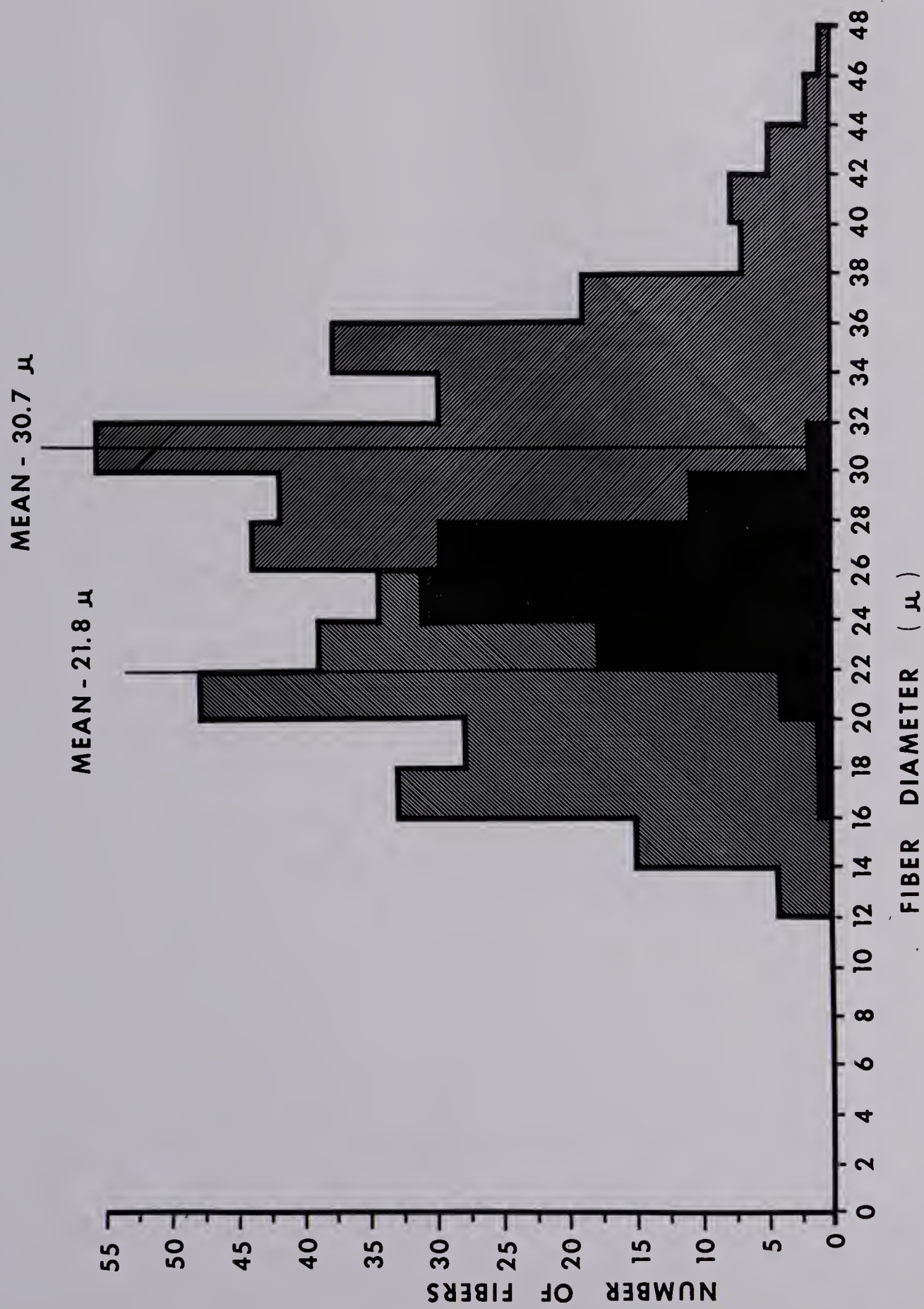


FIG. 6

Fig. 7. Extrafusal (vertical hatching) and intrafusal (nuclear chain - oblique hatching, and nuclear bag - mottled shading) muscle fiber diameter distribution of muscles embedded in paraffin and stained with hematoxylin and eosin.

The histograms are plotted using values uncorrected for shrinkage.

The bracketed figures are the means corrected for 29% shrinkage (see Fig. 6).

Nuclear chain fiber diameter mean $7.7 \mu (\pm 0.97)$; $n = 26$. Nuclear chain fiber diameter mean $10.0 \mu (\pm 1.6)$; $n = 24$. Mean of the extrafusal muscle fiber diameter distribution is $21.8 \mu (\pm 1.4)$; $n = 244$. n = number of fibers measured.

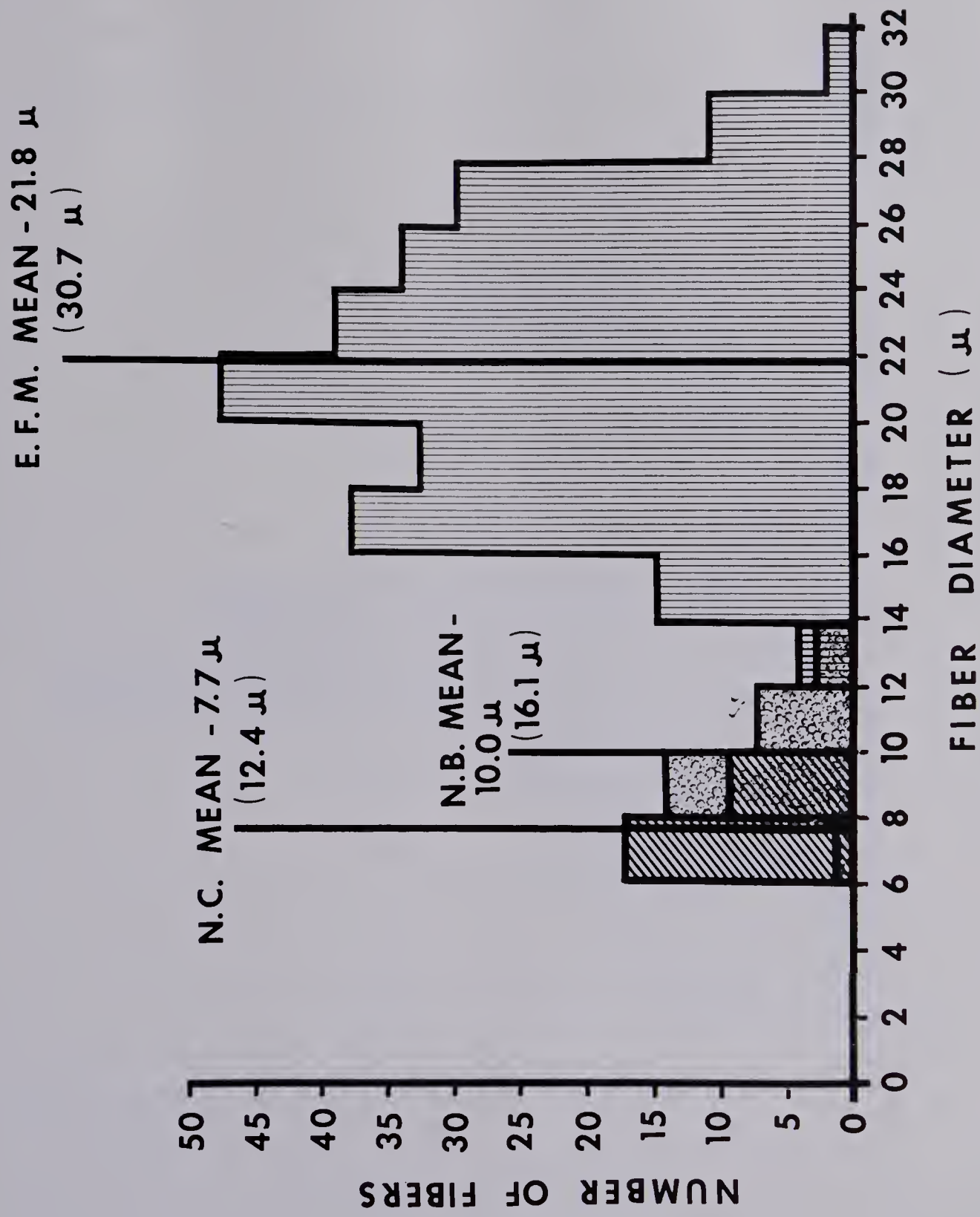


FIG. 7

Fig. 8. Muscle spindle length distribution obtained from measurements of 14 spindles reconstructed from serial cross sections of paraffin embedded material (mottled shading) and measurements of spindle whole mounts stained with Cole's gold chloride method (vertical hatching). All muscles fixed relaxed.

Spindle length mean of paraffin embedded material 1.5mm (± 0.2);

n = 14. Mean of whole mounts stained with gold chloride 3.32 mm (± 1.3);

n = 44. n = number of spindles measured.

Not corrected for shrinkage.

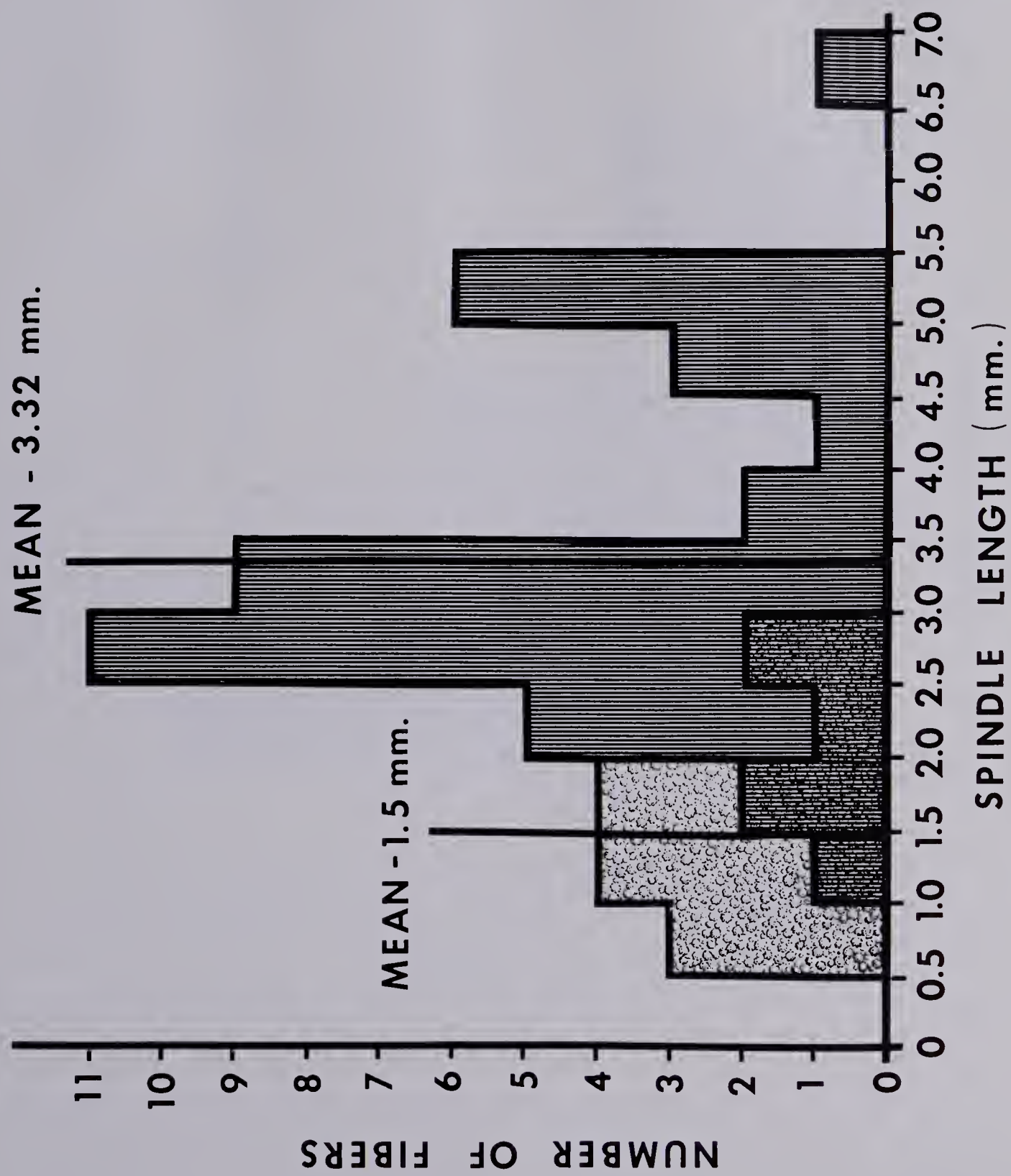


FIG. 8

Fig. 9. Intrafusal muscle fiber length histogram. Data obtained from 14 spindles reconstructed from serial cross sections of hindpaw plantar lumbrical muscles fixed in glutaraldehyde, embedded in paraffin and stained with hematoxylin and eosin. Mottled shading represents nuclear chain length distribution and oblique hatching represents nuclear bag length distribution.

Mean nuclear chain length 1.20mm (± 0.54); n = 26.

Mean nuclear bag length 1.58mm (± 0.65); n = 23. n = number of fibers measured.

Not corrected for shrinkage.

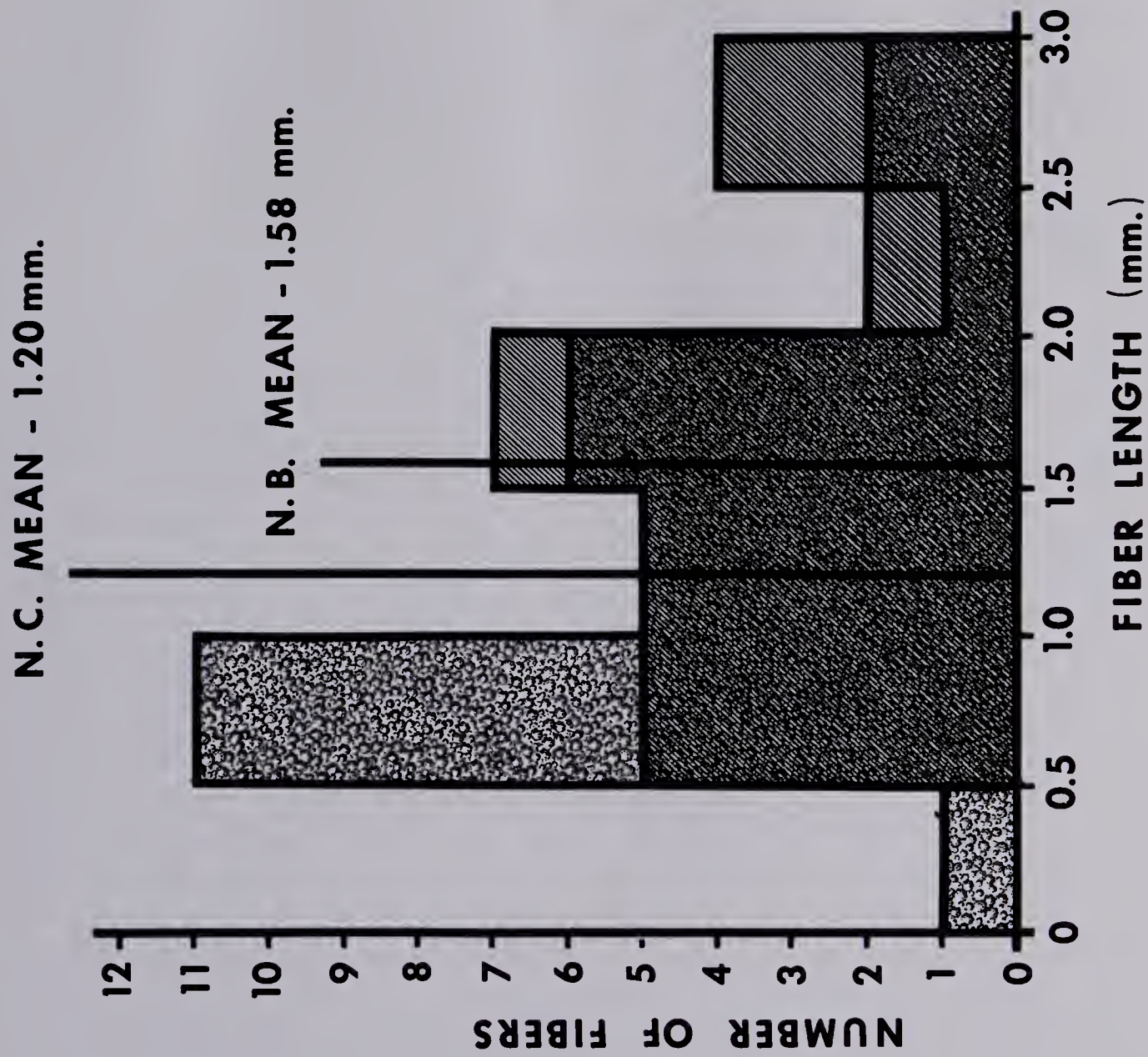


FIG. 9

Fig. 10. Comparison of nuclear dimensions taken from nuclear bag and nuclear chain muscle fibers of the rat and cat. The legend explaining the use of the various symbols is given at the top of the chart.

Note the difference in dimensions of the nuclei of nuclear bag and nuclear chain fibers. Note also the change in nuclear dimensions of nuclei found in the bag region and those found in the myotube region at the polar end of the capsule.

Measurements obtained from spindle whole mounts.

Silver stain.

NUCLEAR DIMENSIONS

- Rat:

- NUCLEAR BAG - BAG REGION
 - NUCLEAR BAG - MYOTUBE REGION
 - ☆ NUCLEAR CHAIN
- Cat:

- x NUCLEAR BAG
 - ★ NUCLEAR CHAIN

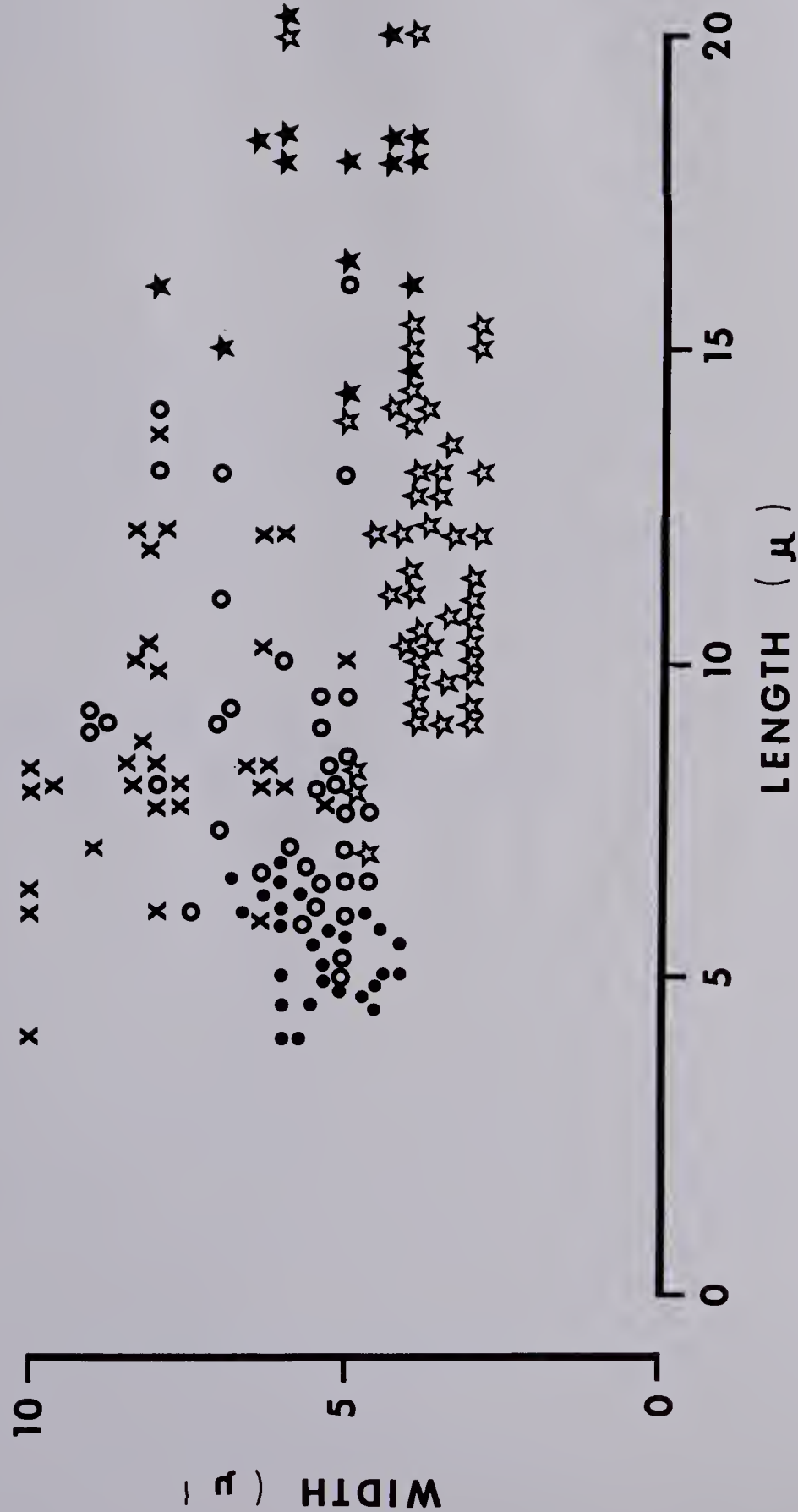


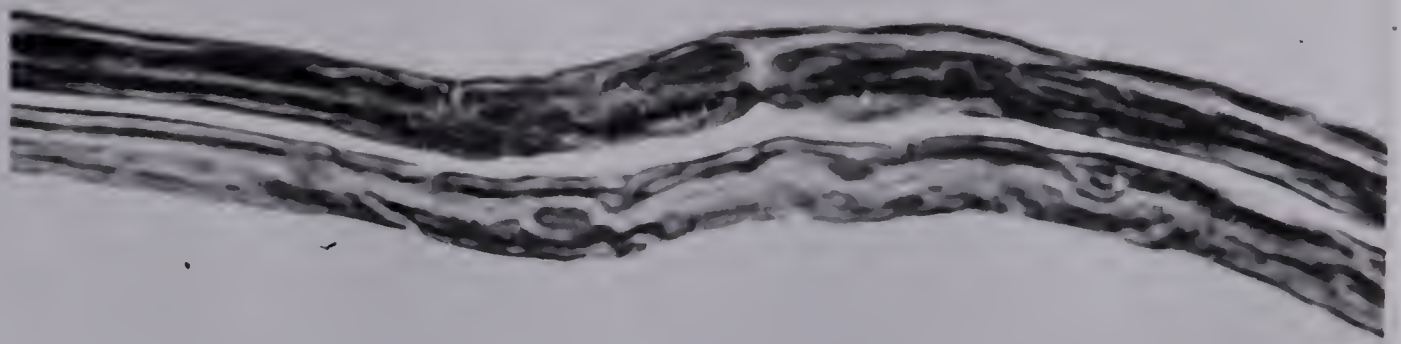
FIG. 10

Fig. II. Whole mounts of myelinated nerve fibers stained with osmic acid at various stages of degeneration.

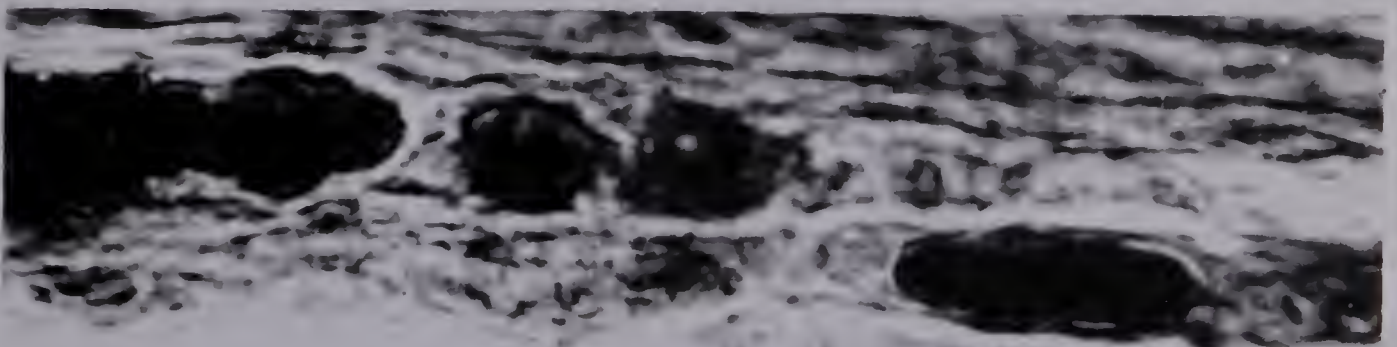
- A. Appearance of normal nerve fibers.
- B. 19 days after spinal root section the myelin has fragmented and appears as swollen droplets.
- C. 32 days following root section myelin is absent and only the Schwann cell tube remains.

All specimens were taken from the tibial nerve.

Scale bar-10 μ .



A



B



C

FIG. 11

Fig. 12. Spindles teased, for control purposes, from muscles attached to the nerve trunks which were used to obtain the cross sections shown in Figs. 13, 14.

A. Spindle in a deafferented preparation. No sensory axons are present.

B. Spindle from a deafferented preparation. Sensory innervation to the spindle is intact, the winding course of the axon can be seen within the spindle capsule. Osmic acid stain. Scale bar-A, 80 μ ; B, 40 μ .

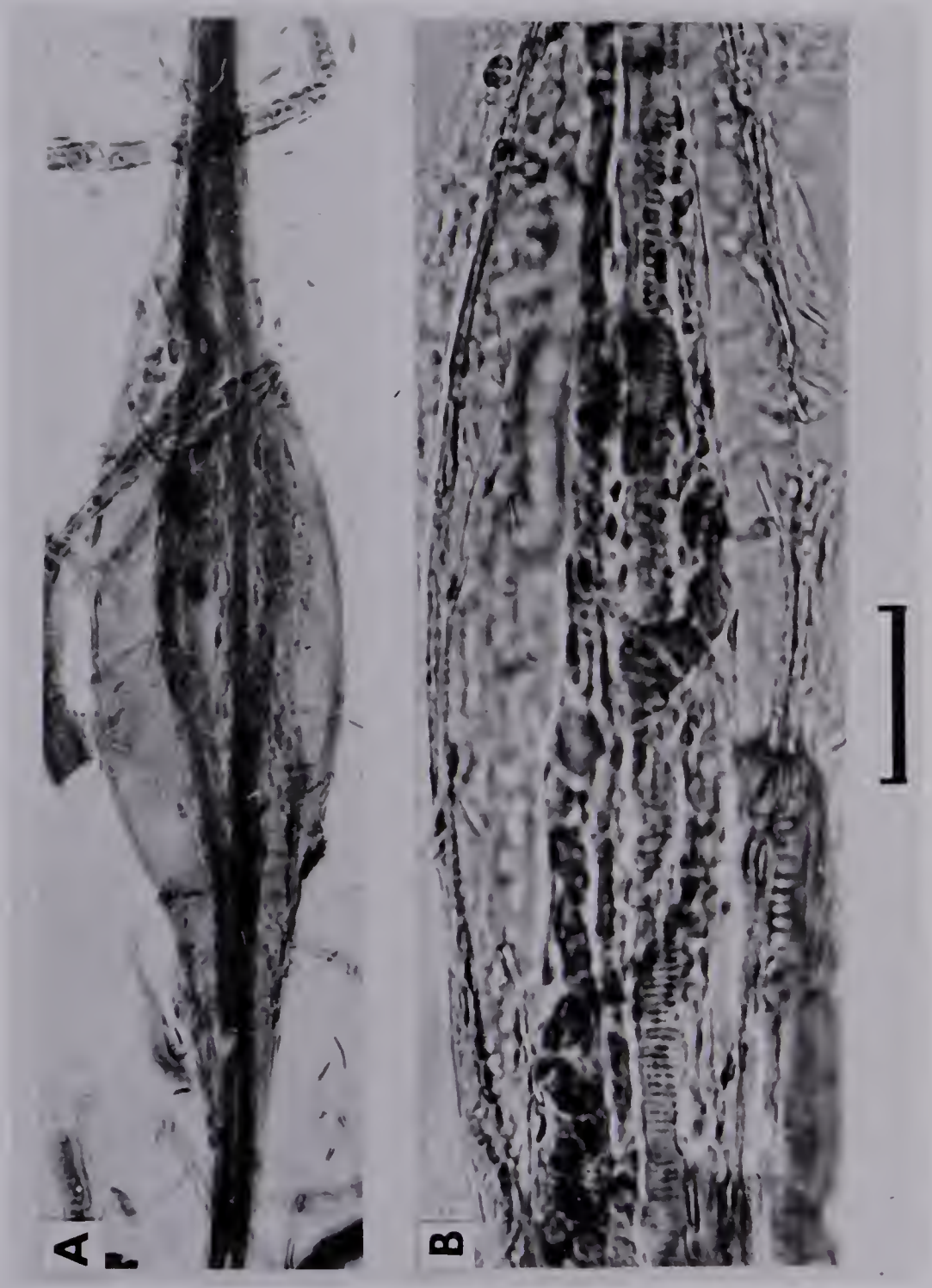


FIG. 12

Fig. 13. Cross sections of the nerve trunk to lumbrical muscle no. 5 with the control sections of the skin nerves to the 5th digit (C and D).

A. shows the appearance of the normal muscular nerve and C the normal skin nerve.

B. shows the muscular nerve 35 days after ventral root section of spinal segments L 4, 5, 6. The skin nerve maintained the appearance shown in C.

D. is the control skin nerve taken from the foot of the same animal as the sections shown in Fig. 14. This animal had undergone dorsal root ganglionectomy of lumbar segments 4, 5, 6 35 days previously. Note the absence of myelinated nerve fibers and the reduced diameter of the nerve.

0.5 μ sections cut from osmic acid-stained, Araldite-embedded material.

Scale bar - A and B, 10 μ ; C and D, 50 μ .

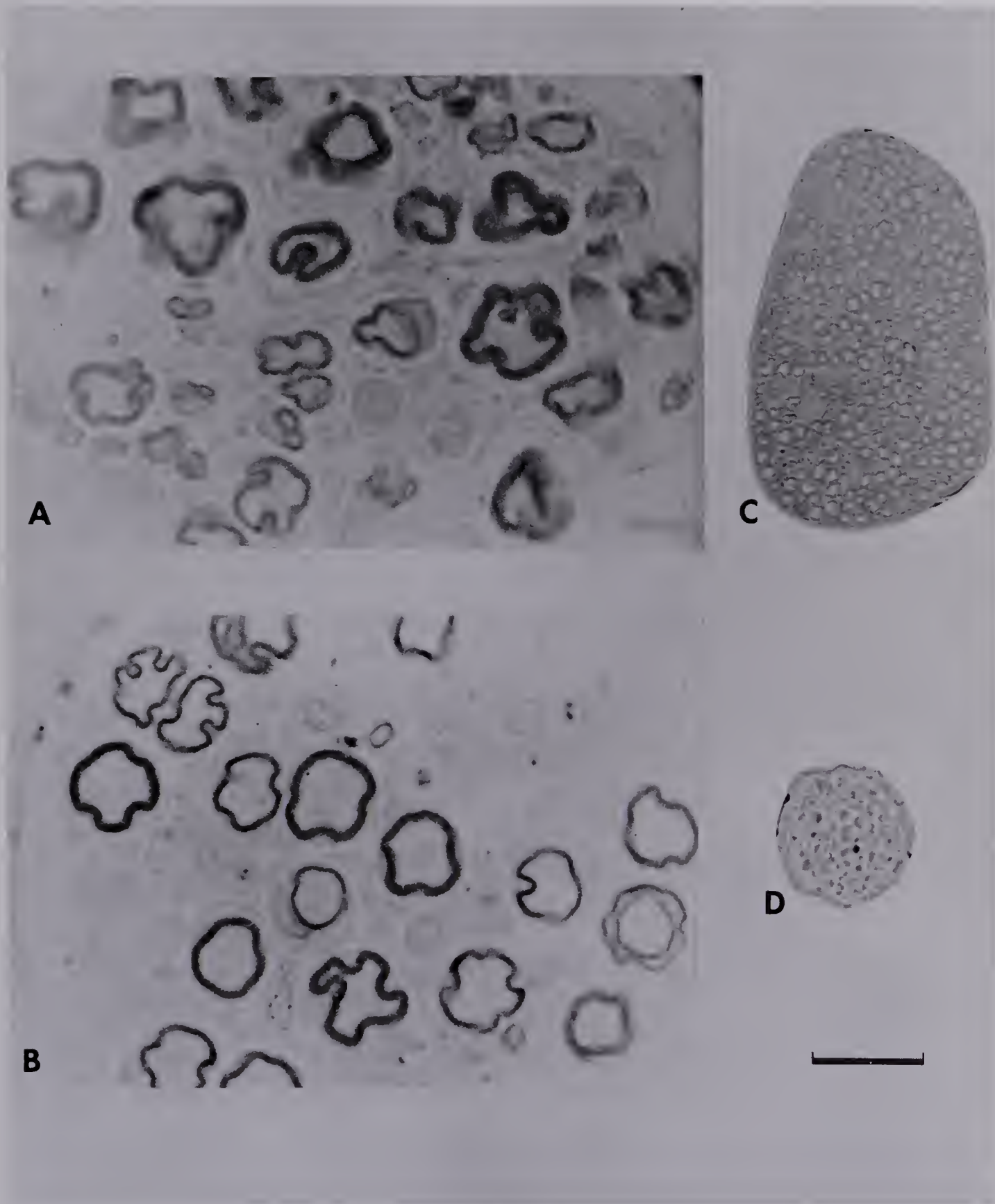


FIG. 13

Fig. 14. Cross sections of myelinated motor nerve fibers. (A) and (B) are adjacent ultrathin (silver) and thin (0.5μ) sections taken from the nerve to lumbrical muscle 5 at 35 days following dorsal root ganglionectomy of spinal segments L 4, 5, 6.

In this case the appearance in the light microscope (B) was low in contrast, however low-power electron microscopy (A) indicated that no myelinated fibers were missed when using light microscopy. (C) shows, for comparison, a section of ventral root L6. Thickly and thinly myelinated small motor nerves can be seen. Osmic acid stain, Araldite embedding.

Scale bar — A, 5μ ; B and C, 10μ .

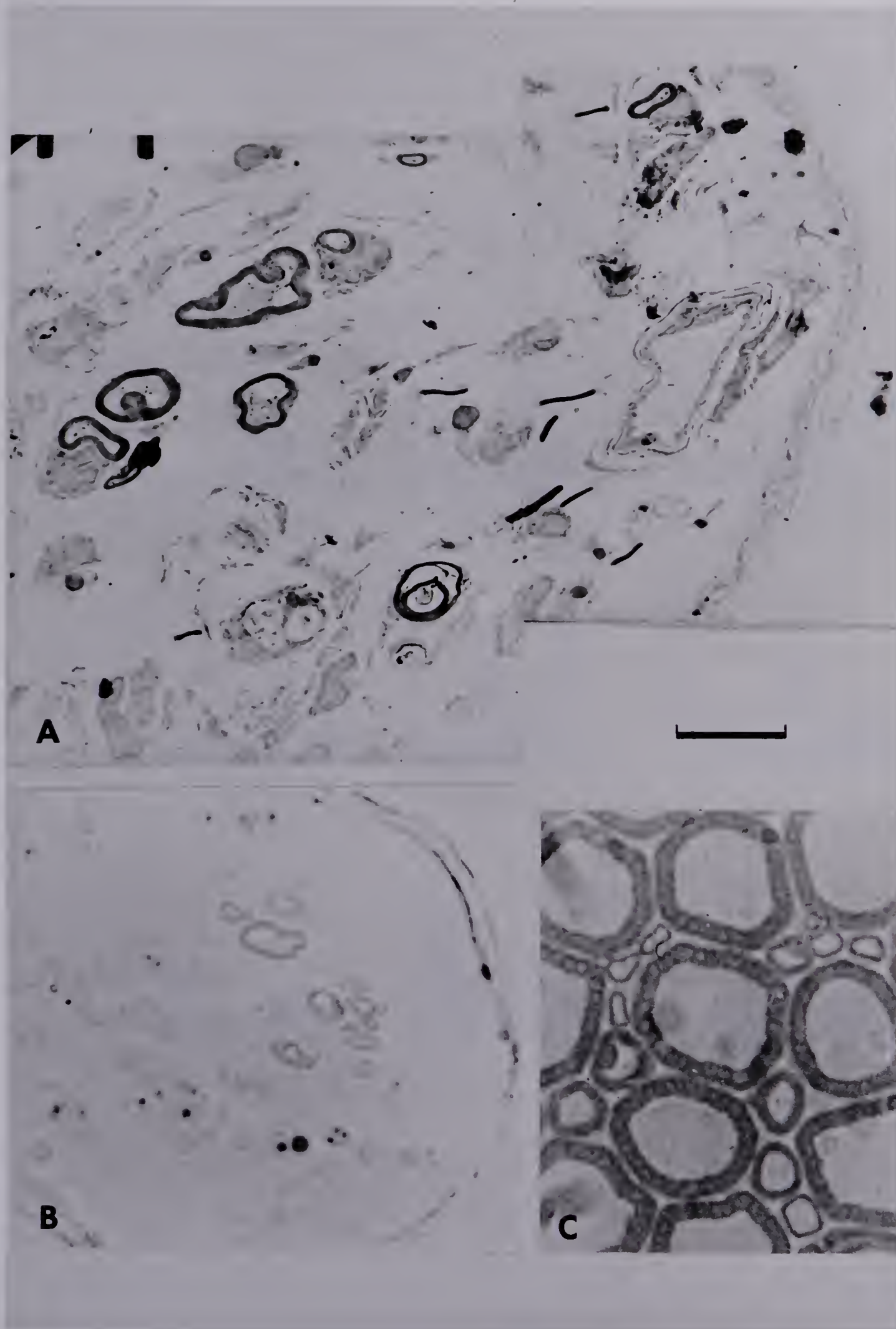


FIG. 14

Fig. 15 (a) (b). Nerve fiber diameter distributions of muscle nerves innervating the lateral central lumbrical muscle (L.C.), medial central lumbrical muscle (M.C.) and lumbrical muscles to the 2nd, 3rd, 4th, and 5th digits (2, 3, 4, 5 respectively). Total nerve fiber diameter values were pooled from one normal animal, two animals deafferented over 35 days and two animals deafferented over 35 days.

Distribution of fibers in normal nerves to these muscles is indicated in the column of histograms headed by "normal". Motor fiber diameter distribution is indicated in the column of histograms labelled "deafferented" and sensory fiber diameters in the third column labelled "deafferented".

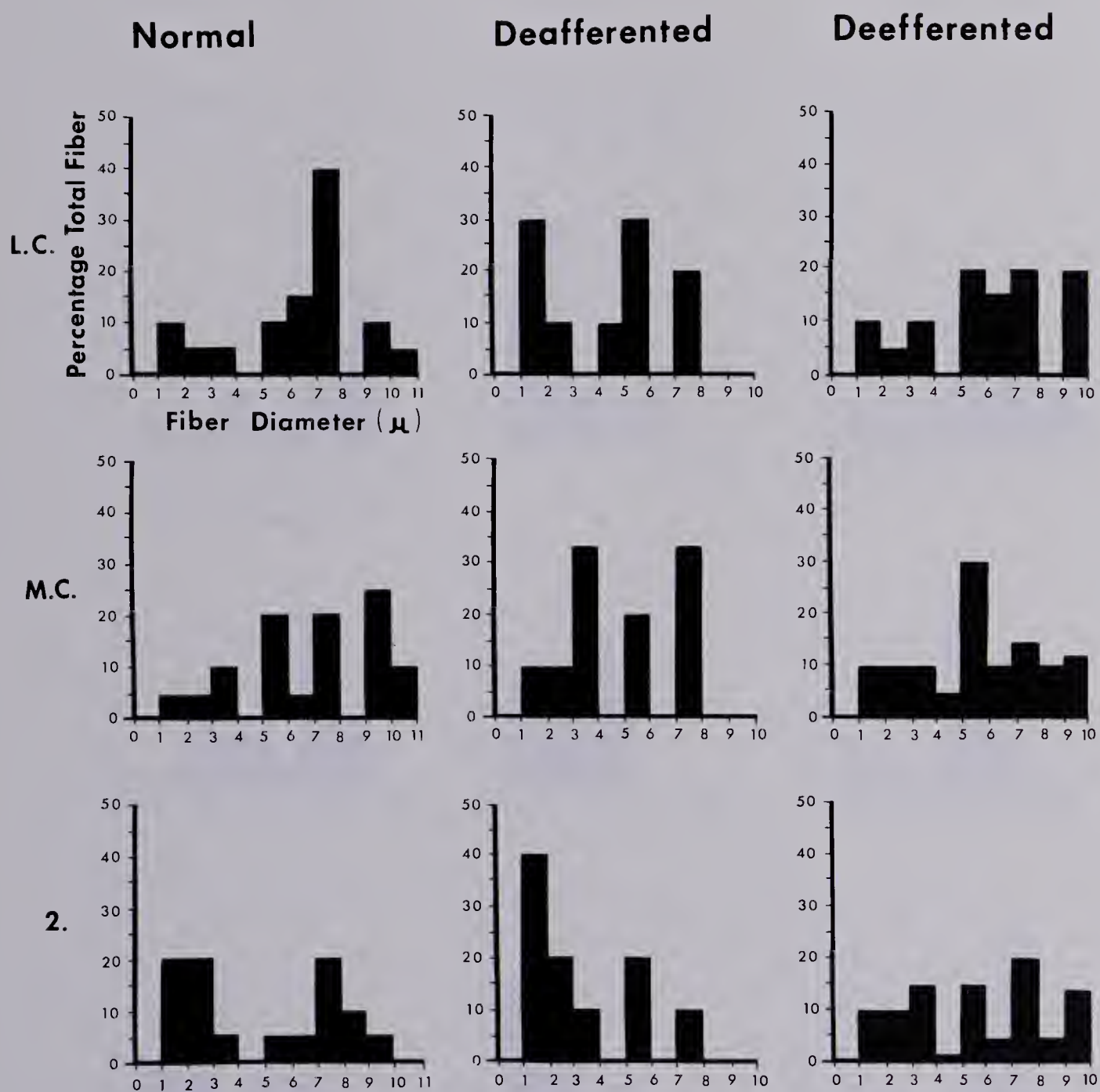


FIG. 15 (a)

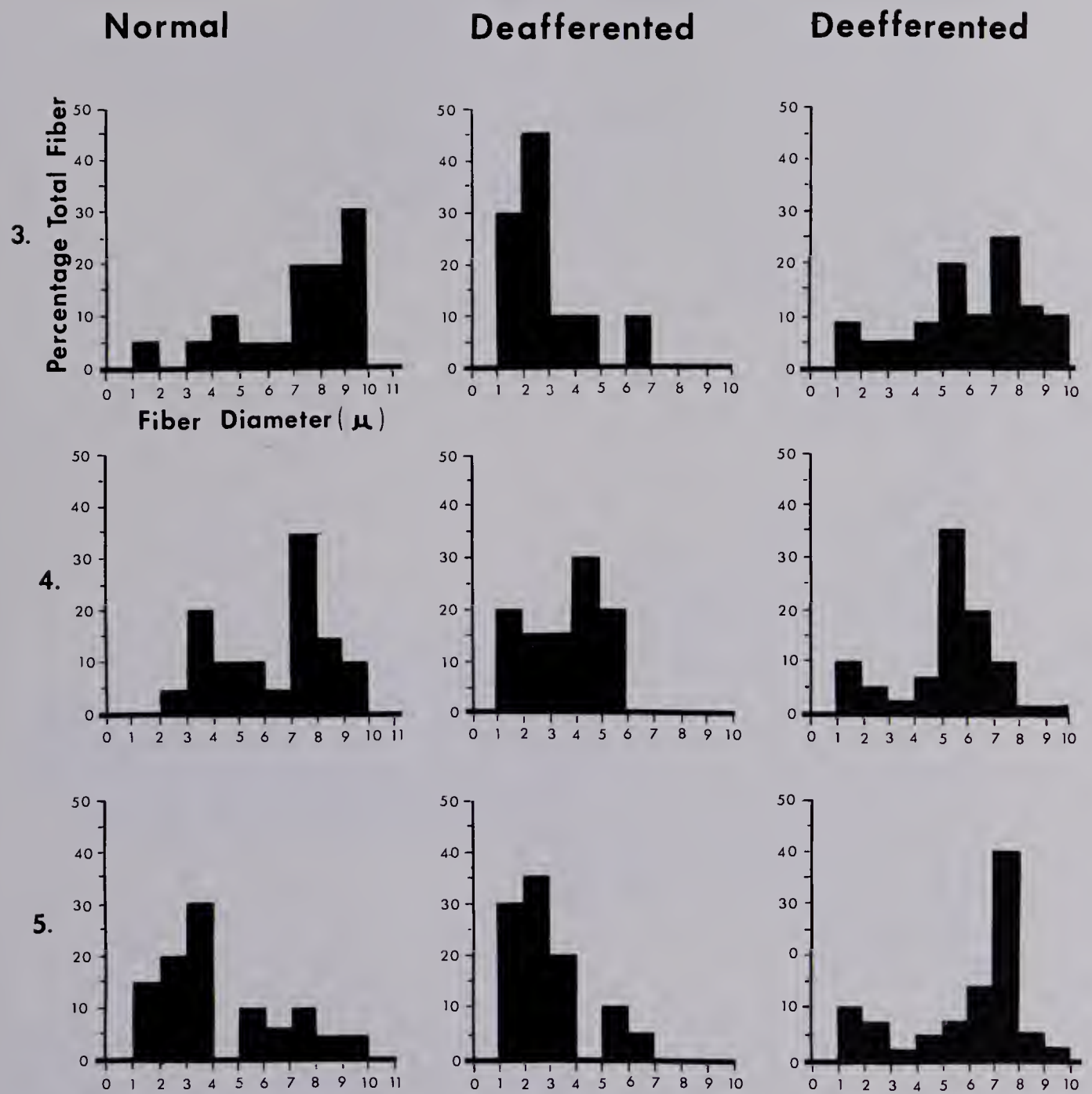


FIG. 15 (b)

Fig. 16. Histogram showing distribution of a random sample of nerve fibers contained in lumbar ventral roots 4, 5, 6. Measurements include the myelin sheath. Specimens fixed in glutaraldehyde, stained with osmic acid and embedded in araldite.

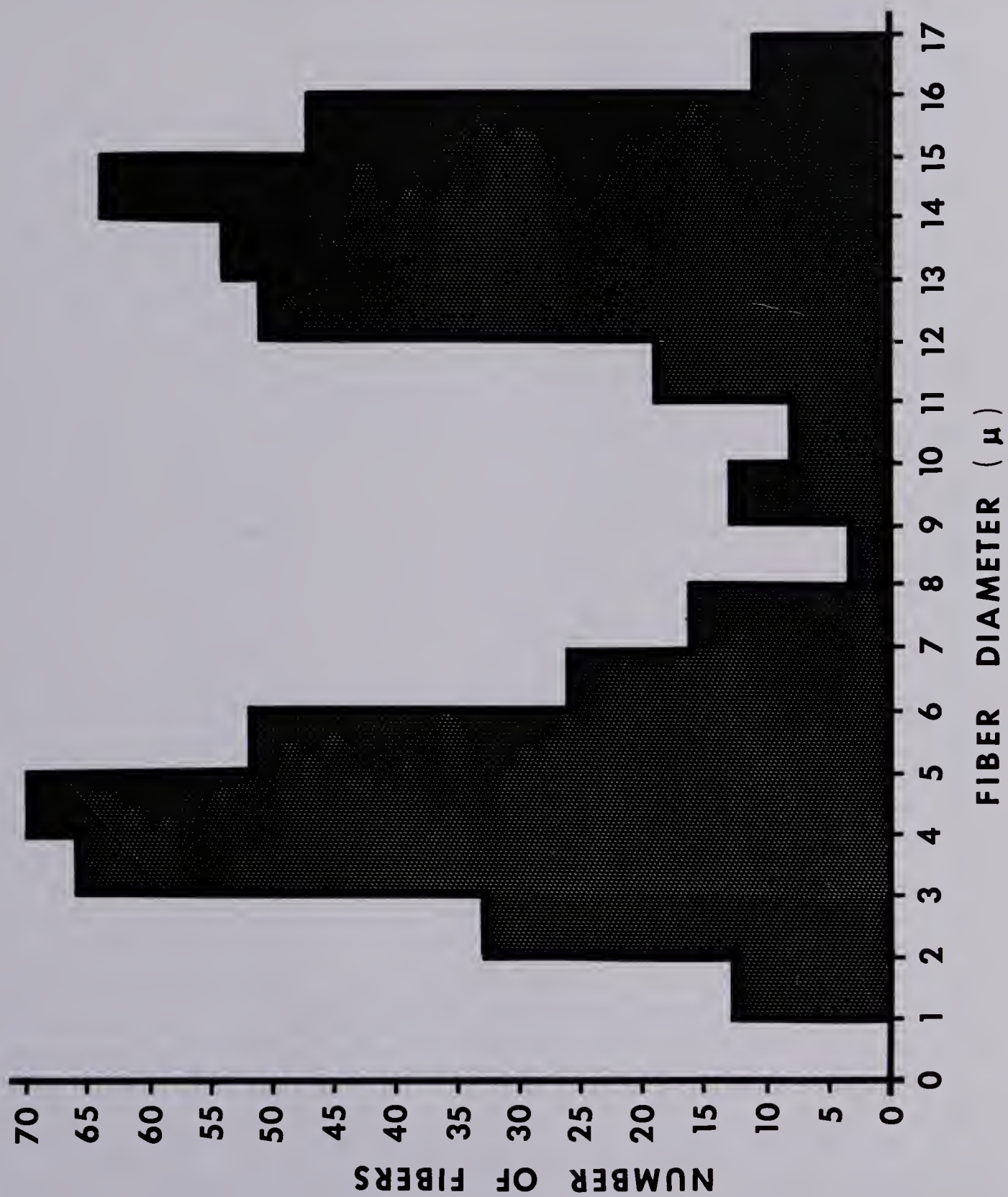


FIG. 16

Fig. 17. Schematic representation of the apparatus in which stimulating and recording procedures were carried out on the nerve (A) and nerve-muscle (B) preparations. The recording chamber consisted of 2 blocks of Perspex glued together. The lower block contained a zig-zag enclosed groove through which water flow allowed for temperature regulation. The upper Perspex block had a longitudinal groove in which Ringer's solution and the nerve specimen (A) or the nerve-muscle preparation (B) could be placed. Temperature of the Ringer's solution was constantly monitored by the thermistor thermometer. The sites of the stimulating and recording electrodes are indicated. The center compartment was grounded. The partitions were moveable and allowed adjustment for nerves of different lengths as well as stimulation of the same nerve specimen at different sites (1 and 2). The inset (B) shows a modification of the recording end of (A). One tendon was held by a nylon rod mounted in the side of the chamber. The other tendon was attached to a strain guage.

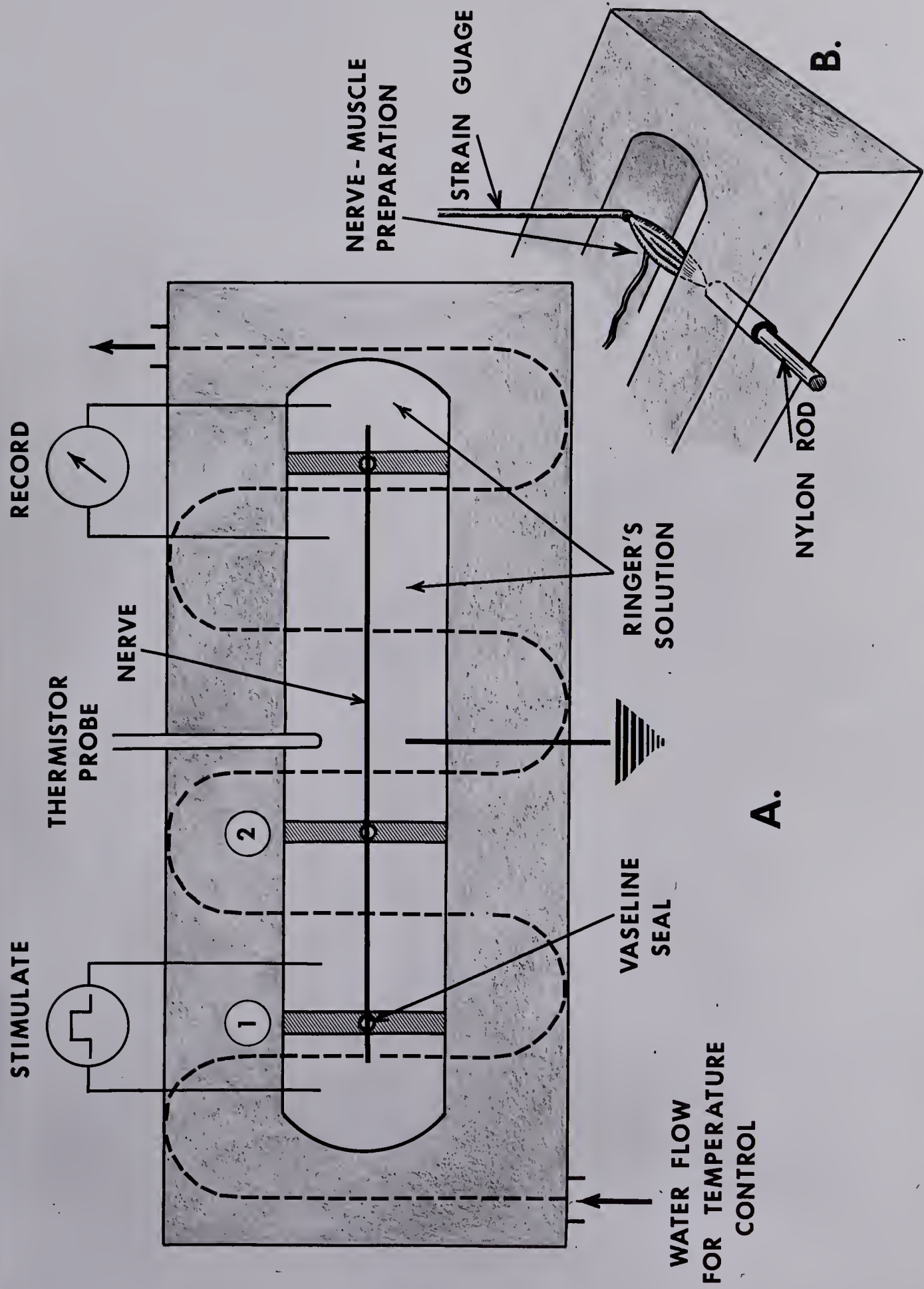


FIG. 17

Fig. 18. Conduction characteristics of nerve fibers contained in the nerve to lumbrical muscle 5.

A to C shows the effect of temperature variation on the nerve conduction velocity. Recordings taken from the nerve specimen in the recording chamber as shown in Fig. 17. Conduction distance 7.0 cm.

D to F are recordings, taken in vivo, from the nerve to lumbrical muscle 5 after stimulating L6 dorsal and ventral spinal roots.

D is the compound action potential recorded after stimulating dorsal and ventral roots together. E and F show the sensory and motor components respectively. Conduction distance 13.8 cm. Rectal temperature 28 C.

The graph illustrates the temperature dependence of the conduction velocity of selected components of the compound action potential.

I, II and III are measurements taken from peaks within the compound action potential, (I) being the fastest group of fibers. Conduction velocities were obtained from the leading edge of the compound action potential by stimulating at one site only.

The difference between regression lines I and Ia indicates that measurements made with one stimulating site only must be corrected. (s) and (m) are the corrected fastest sensory and motor conduction velocities obtained by stimulating L6 dorsal and ventral spinal roots respectively in several animals.

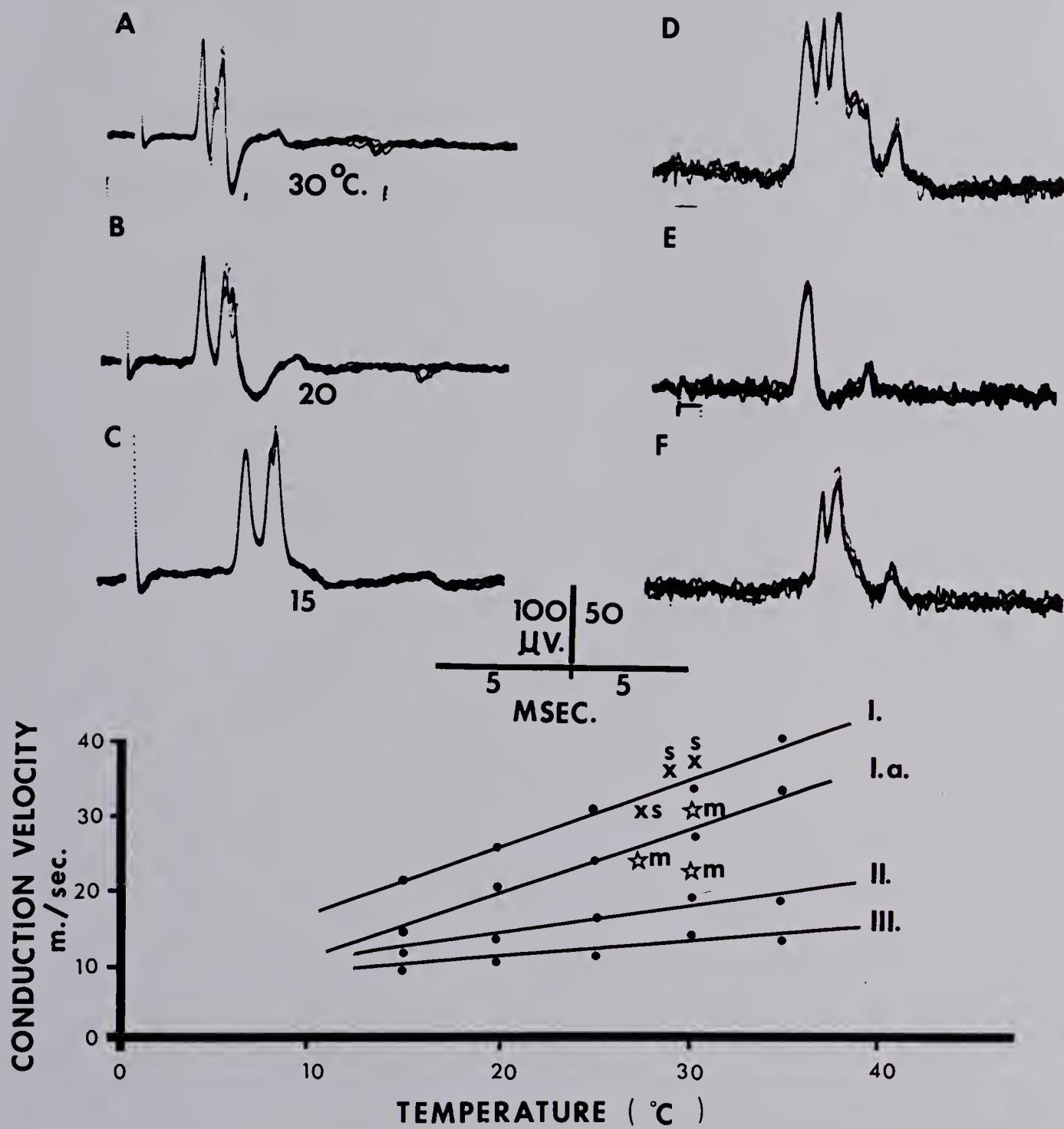


FIG. 18

Fig. 19.

- A. Contractile responses of lumbrical muscle 5 on increasing the stimulus strength to the motor nerve trunk. The stimulus strength increases from top to bottom.
- B. Response of lumbrical muscle 5 to a supramaximal stimulus applied to the nerve trunk. The stimulus frequency increases top to bottom from 20-100 imp./sec. in increments of 20/sec.

Temperature 32.5°C.

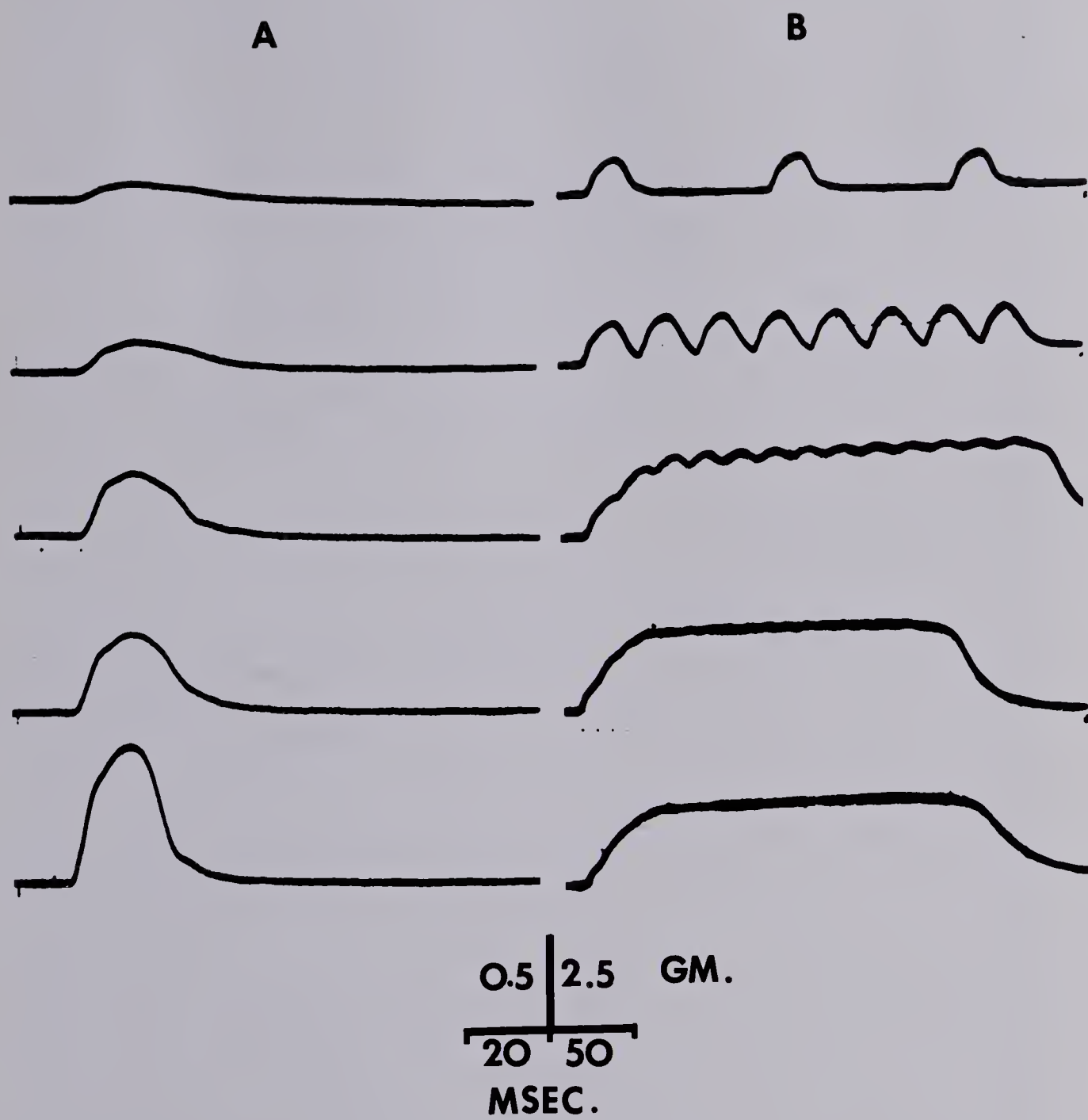


FIG. 19

Fig. 20. Three examples of beta or skeletofusimotor innervation.

A. An axon enters at the top right of the photograph and divides at the arrowhead.

One branch of the axon ends as a plate (p) on a nuclear bag muscle fiber (nb).

The other branch innervates an extrafusal muscle fiber (efm) through a group of motor end-plates.

B. A full arrow shows the direction of entry of two motor axons, (1) and (2), from the left-hand side of the photograph. (1) is a pure fusimotor axon and continues in the direction of the full arrow on the right-hand side of the photograph to innervate a nuclear chain muscle fiber. Axon (2) branches at the arrowhead giving rise to axons (3) and (4). Axon (3) terminates as an end-plate on a nuclear bag muscle fiber (not shown). Axon (4) innervates an entrafusal muscle fiber (not shown).

C. Axons (1) and (2) enter the top left-hand side of the photograph. Axon (1) innervates an extrafusal muscle fiber at the bottom left-hand side of the photograph. Axon (2) divides at the arrowhead into branches (3) and (4). Branch (3) innervates an entrafusal muscle fiber (not shown). Branch (4) terminates as a plate-ending (p) on a nuclear bag fiber of the muscle spindle (sp).

Silver stain. Scale bar — A and B, 20 μ ; C, 40 μ .

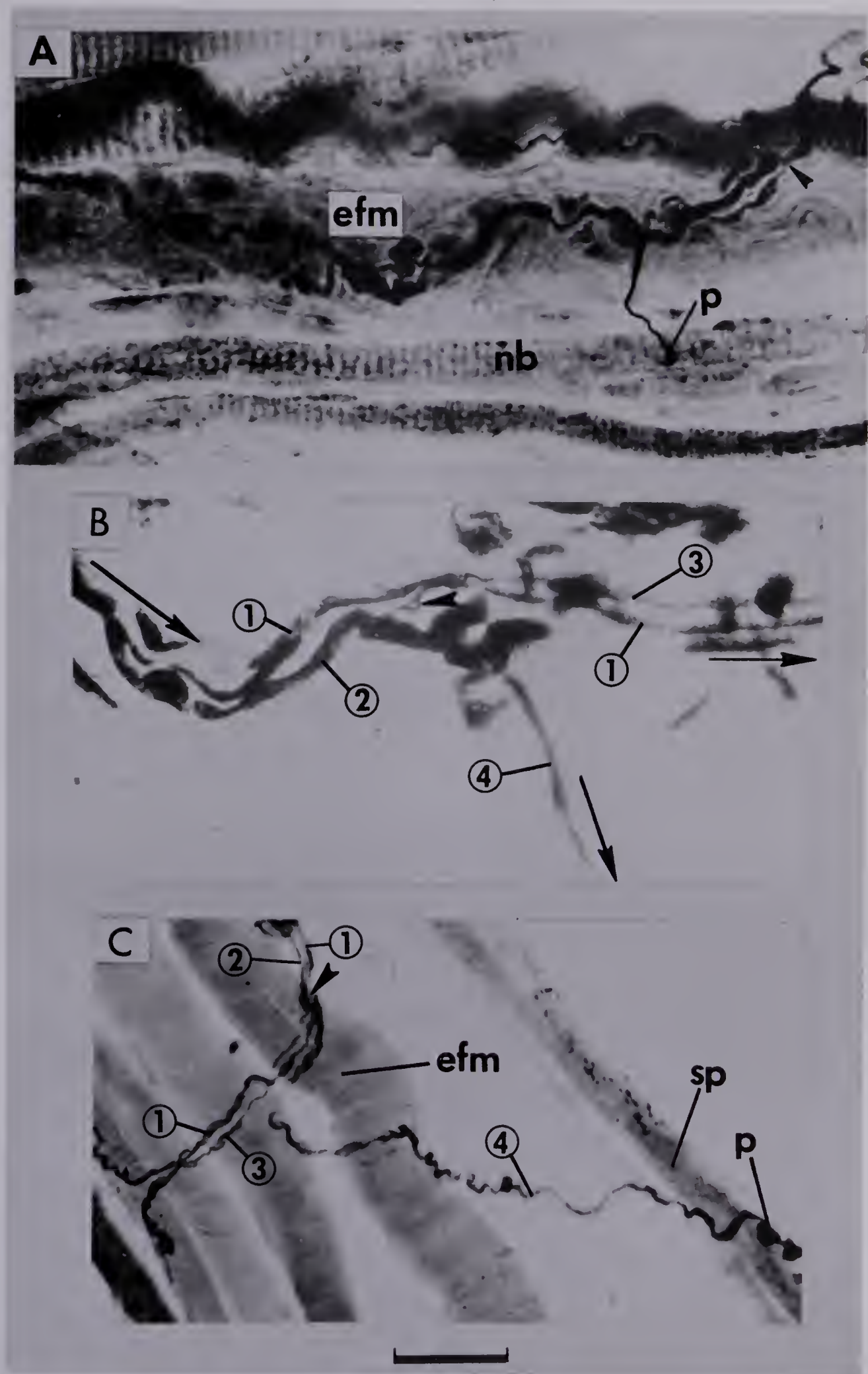


FIG. 20

Fig. 21. An illustration of a fusimotor axon branching to innervate adjacent spindles.

- A. Shows the capsular region of a spindle (sp) with sensory axons to primary (1°) and secondary (1 1) endings. Entering the capsule with the axon to the primary ending is a fusimotor axon (m) which exists the photograph to the left in the direction of the arrow.
- B. The fusimotor axon (m) seen in (A) enters the right hand side of the picture in the direction indicated by the arrow. It divides, in the midpolar region, at the arrowhead to terminate on a nuclear bag fiber (nb) of spindle (sp) as 2 end-plates. The capsular region of an adjacent spindle (sp') lies above and to the left of the polar end of the lower spindle (sp).
- C. The fusimotor axon (m in A and B) branches at the arrowhead and continues as m' into the capsule of spindle sp' to innervate the intracapsular portion of a nuclear bag fiber (nb) as indicated by the cross (+).
- D. The cross (+), seen also in (C), marks the location of innervation of the intracapsular portion of a nuclear bag fiber (nb) by the fusimotor branch (m') to spindle sp'.

Silver stain. Scale bar — A and C, 40 μ ; B and D, 20 μ .

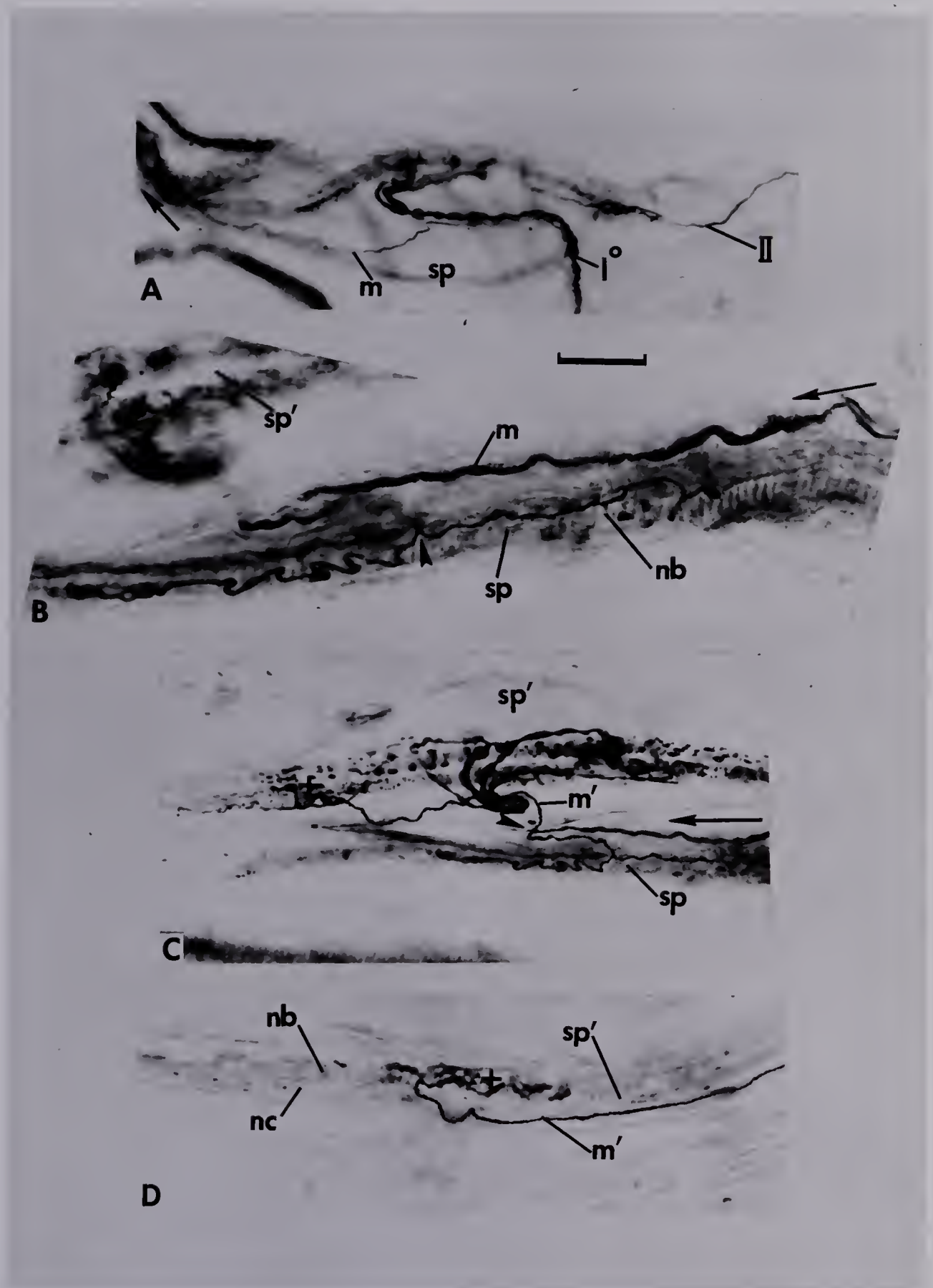


FIG. 21

Fig. 22. Silver - stained whole mounts of muscle spindles from the external intercostal muscle of the cat to show the quality of axon staining.

- A. Secondary sensory endings (I I).
- B. Spiral sensory endings on nuclear chain muscle fibers (nc).
The nuclear staining also identified the compact nuclei of a nuclear bag muscle fiber (nb).
- C. A plate ending (p) on a nuclear bag muscle fiber (nb).
- D. A fusimotor nerve (m) ending on a nuclear bag muscle fiber (nb)..
- E. A primary sensory ending (1°) branching to supply nuclear bag (nb) and nuclear chain (nc) muscle fibers.

Scale bar — B and C, 20 μ ; A, D and E, 40 μ .

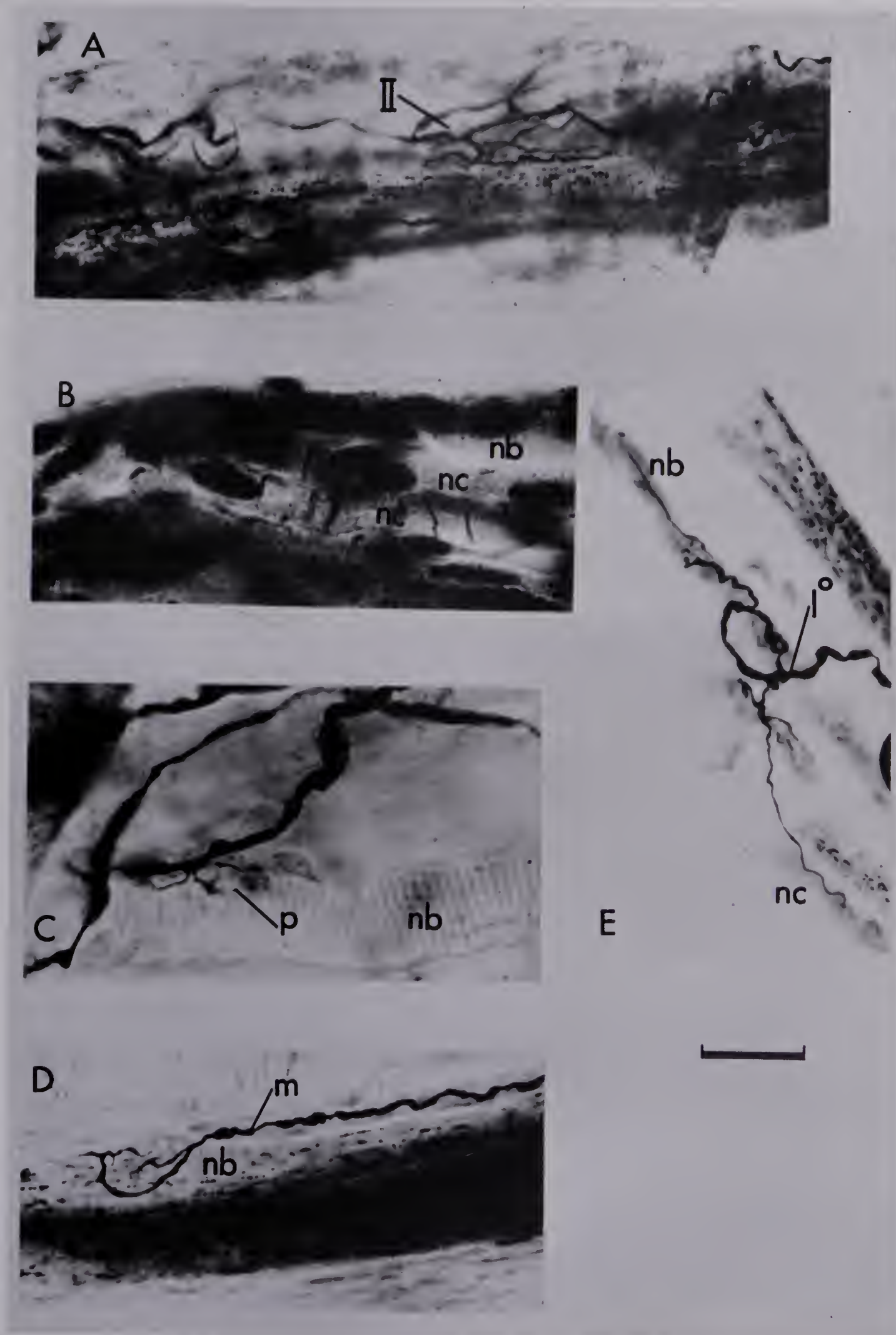


FIG. 22

Fig. 23. Examples of motor - endings.

A and B show motor end-plates on extrafusal muscle fibers. In B an end-plate "sprout" is indicated by the arrowhead. C - I. Fusimotor innervation of the spindle. Note that the innervation of nuclear bag and nuclear chain intrafusal fibers are separate and that no cross motor innervation is present. All motor-endings in this sequence are of the plate variety. Nuclear bag (nb) innervation is shown in C, F, G, I and nuclear chain (nc) in C, D, E, H.

Note that the discrete, compact morphology of the intrafusal motor end-plates is similar to that found on the extrafusal muscle fibers.

Silver stain. Scale bar 40 μ .

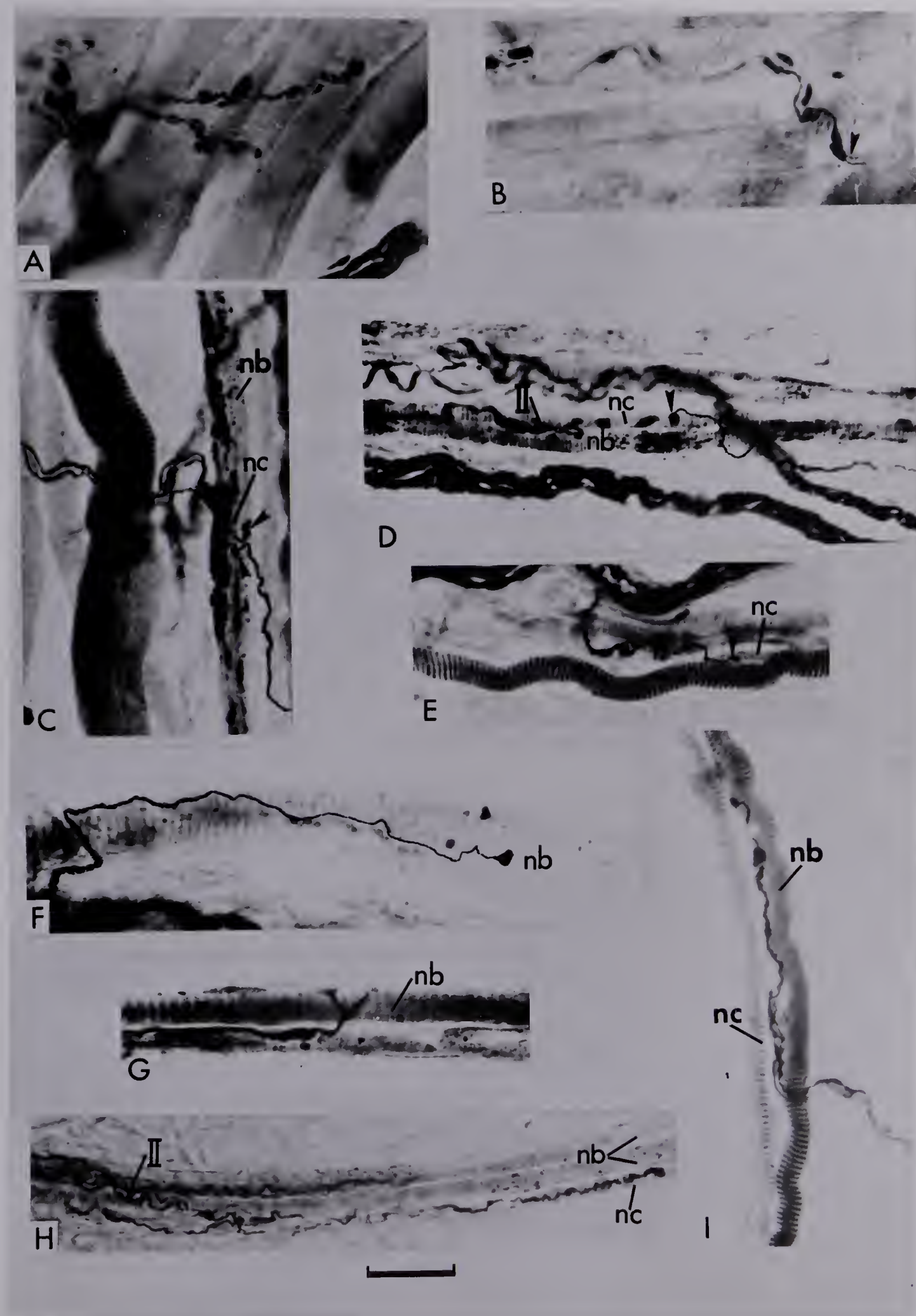


FIG. 23

Fig. 24. Fusimotor innervation at the polar ends of several spindles.

A to F show two varieties of motor-endings as found on both nuclear bag (nb) and nuclear chain (nc) fibers. Compact motor end-plates, as seen in Fig. 23, are present. Several well stained axons seen to terminate, without motor end-plates, on both nuclear bag and nuclear chain fibers. This mode of termination will be referred to as filamentous motor endings. Note that there is no overlapping innervation of nuclear bag and nuclear chain fibers from individual fusimotor axons. Silver stain. Scale bar 40 μ .

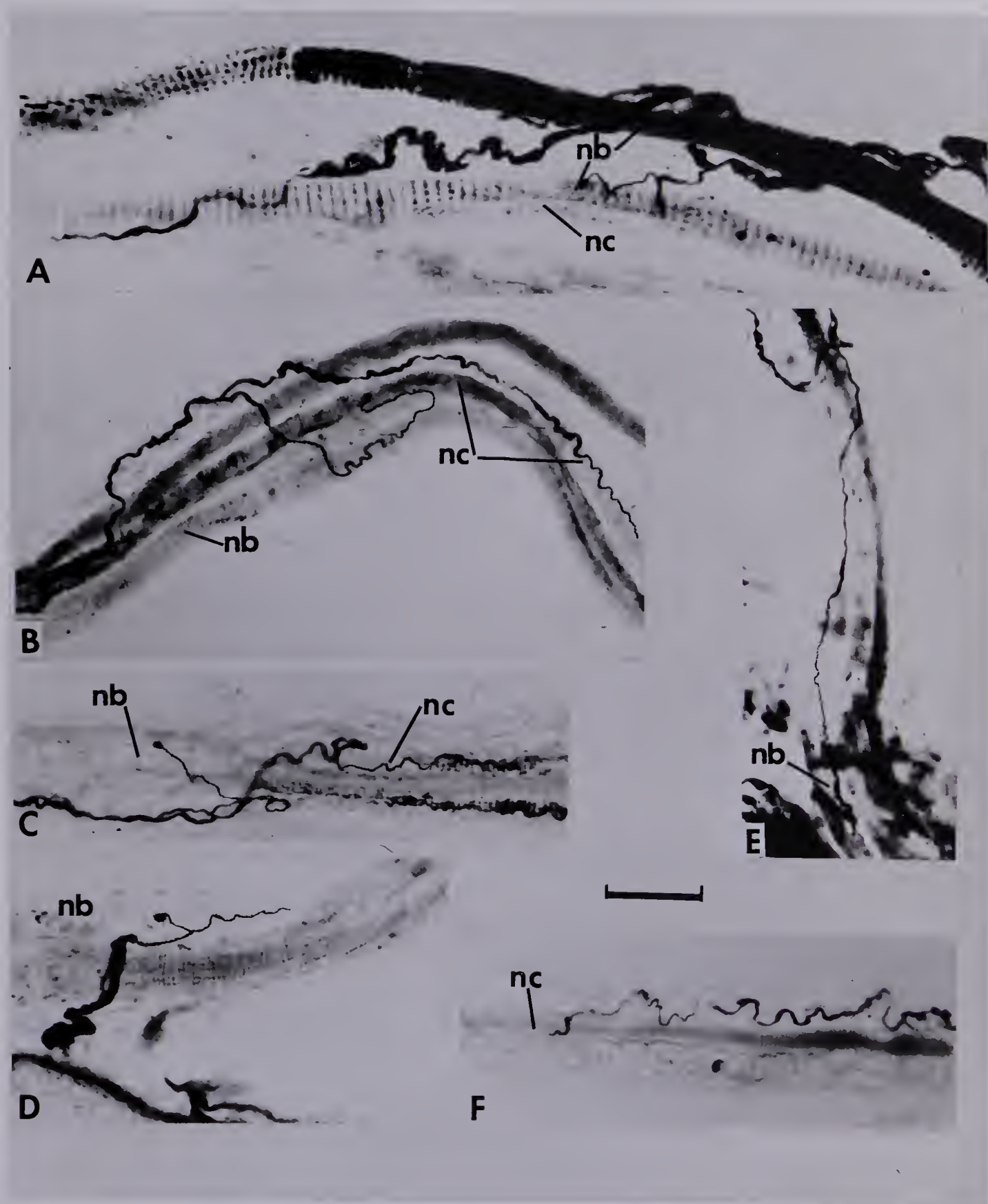


FIG. 24

Fig. 25. The entire motor innervation to one pole of a spindle. A diagrammatic reproduction of the innervation shown here is found in Fig. 26 (a).

Two motor axons, one thick and one thin, enter from the capsular region of the spindle (not shown) on the left-hand side of the photograph. The thick axon branches in the midpolar region to innervate a nuclear bag fiber (NB) as a filamentous ending. The original thick motor axon then continues, in company with the thin axon, toward the polar end of the spindle where the thin axon branches to end as end-plates on 2 nuclear chain (NC) fibers and the thick axon continues, passing beneath an extrafusal muscle fiber (EFM) which partially overlies the spindle pole, and terminates as 2 end-plates on a nuclear bag fiber (NB).

7 days deafferented. Silver stain. Scale bar 50 μ .

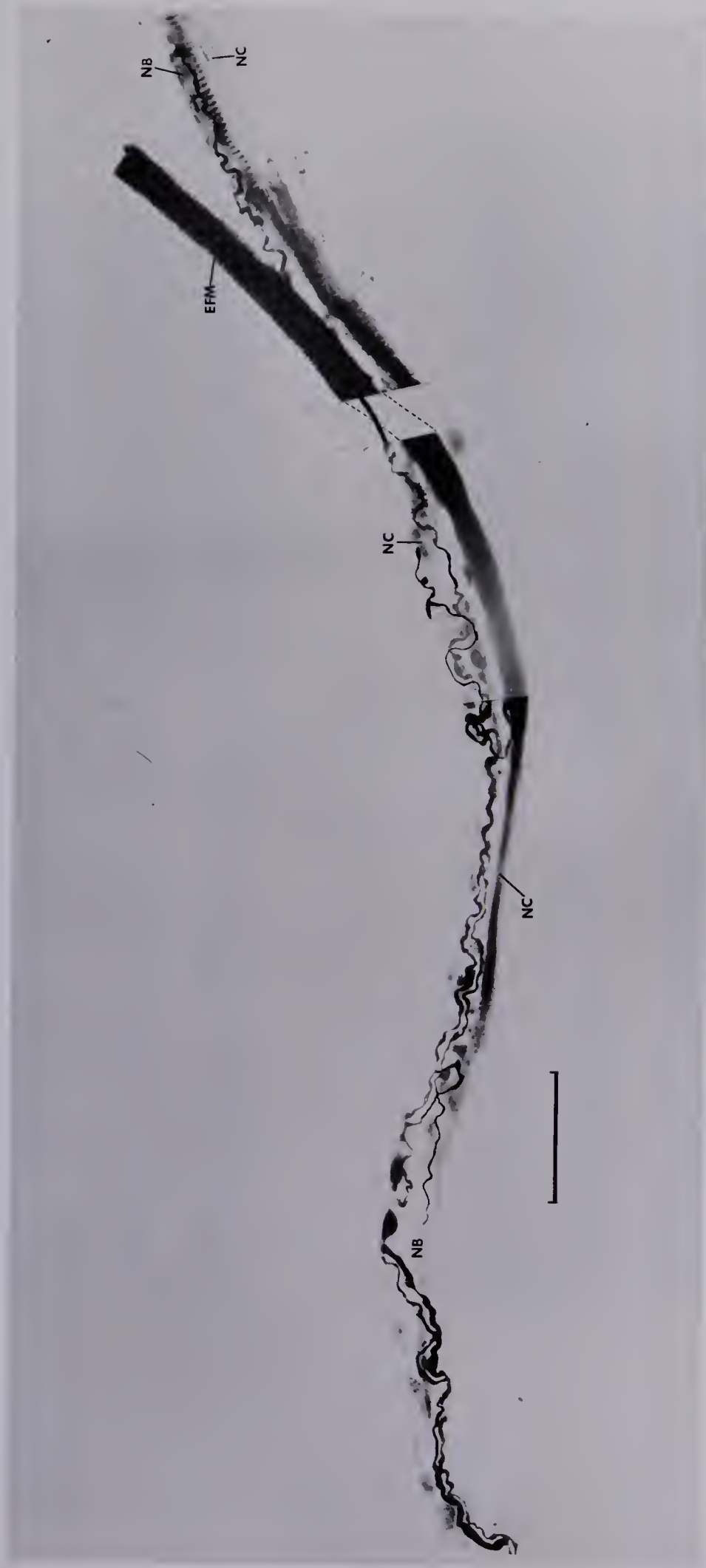


FIG. 25

Fig. 26 (a) (b).

A to D. Schematic representations of the motor innervation of four spindles.

A. is the diagrammatic representation of the spindle as seen in Fig. 25.

In all diagrams, 2 fusimotor axons are seen entering the spindle. The innervation to the nuclear bag (N.B.) and nuclear chain (N.C.) muscle fibers is separate in all cases. Motor end-plate and filamentous endings are represented on nuclear bag and nuclear chain fibers. Note that the thick motor axon innervates the nuclear bag fibers in all cases. One nuclear chain fiber in (B) and (D) lacked demonstrable motor innervation. Capsular and polar ends of the spindle are indicated.

All specimens drawn from teased spindles.

Silver stain. 7 days deafferented.

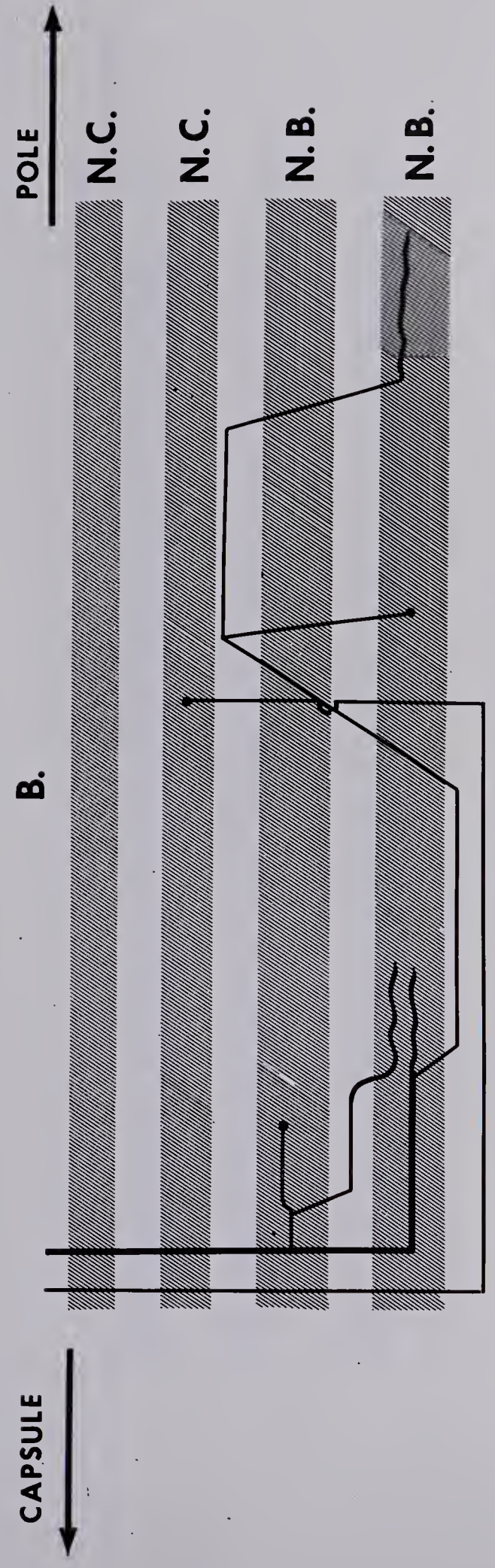
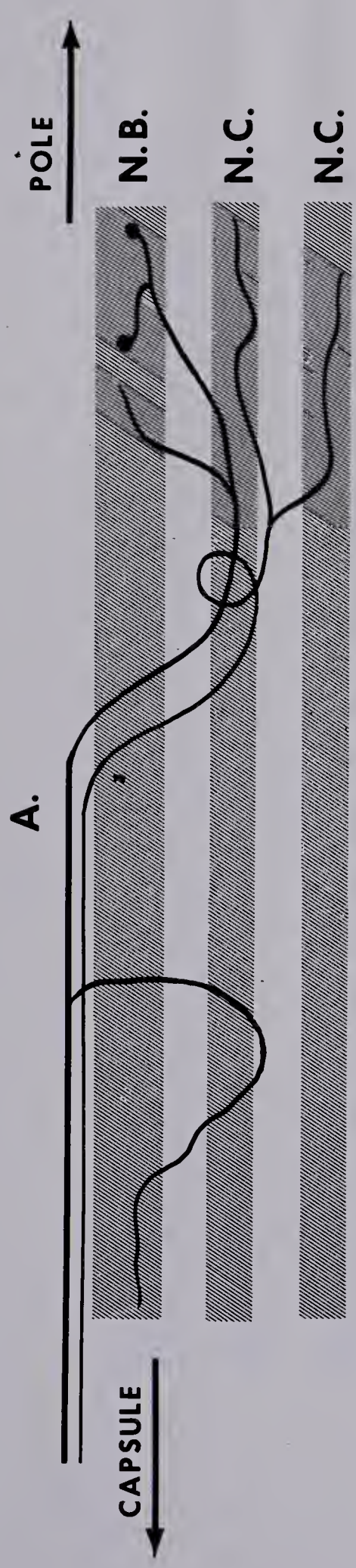


FIG. 26 (a)

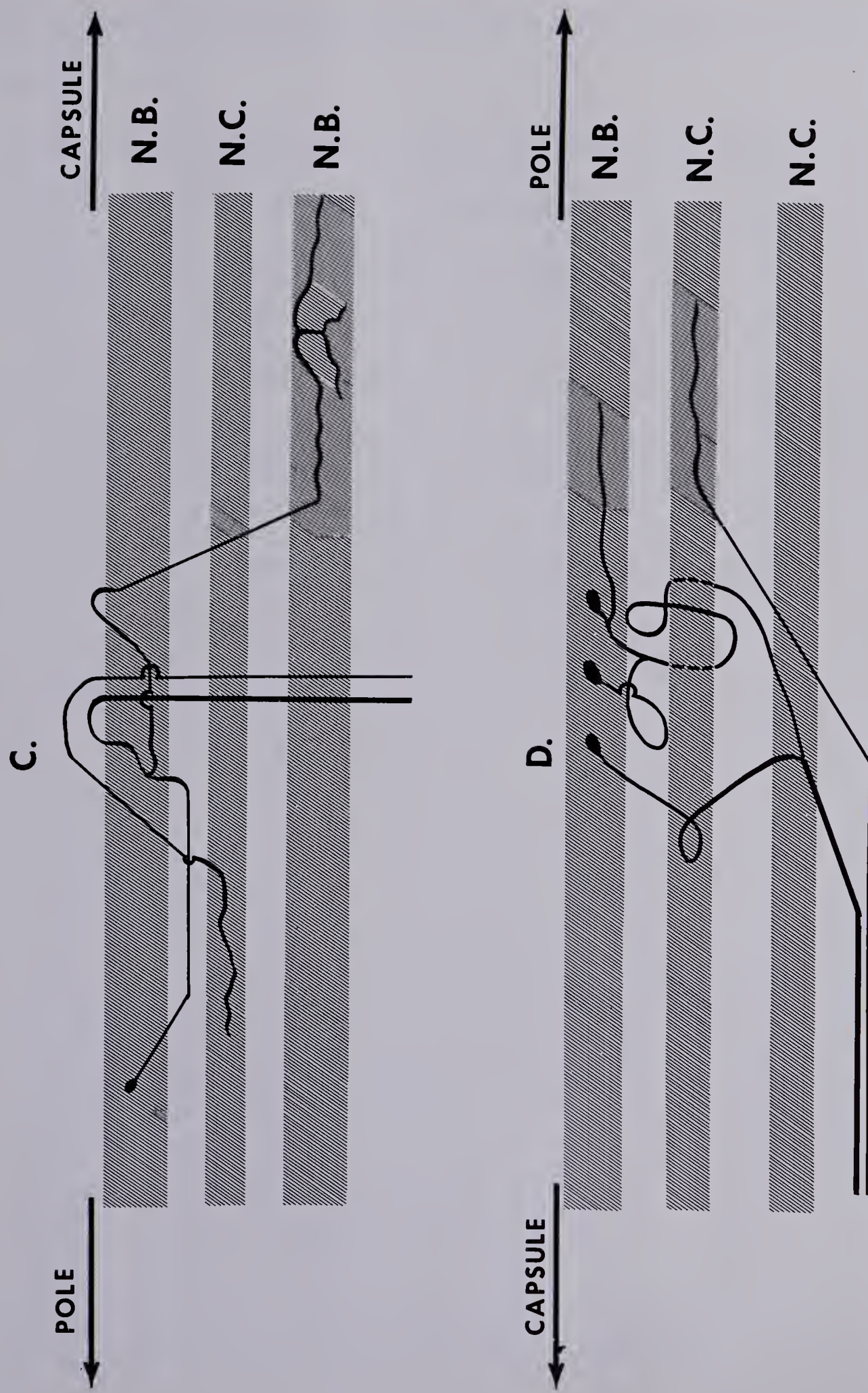


FIG. 26 (b)

Fig. 27. Photomicrographs to show gross appearance and innervation of spindles in the equatorial region.

A. Two sensory axons enter the capsule at the left-hand side of the photograph.

One axon ends as a primary ending (1°), the other sends a branch to the right side of the photograph where it terminates in a secondary ending.

B. The intramuscular nerve trunk crosses the spindle at the left side of the

photograph and sends off axons to the spindle. (l l) indicates a secondary sensory

ending. An axon (1°) terminates as a primary ending. (m) indicates a fusimotor axon which traverses the length of the capsule.

C. A simple spindle. (1°) indicates the axon leading from the primary endings.

D. Primary (1°) and secondary (l l) axons entering the capsule. Note the complex division of the axons.

Silver stain. Scale bar — A, B, C, 80 μ ; D, 40 μ .

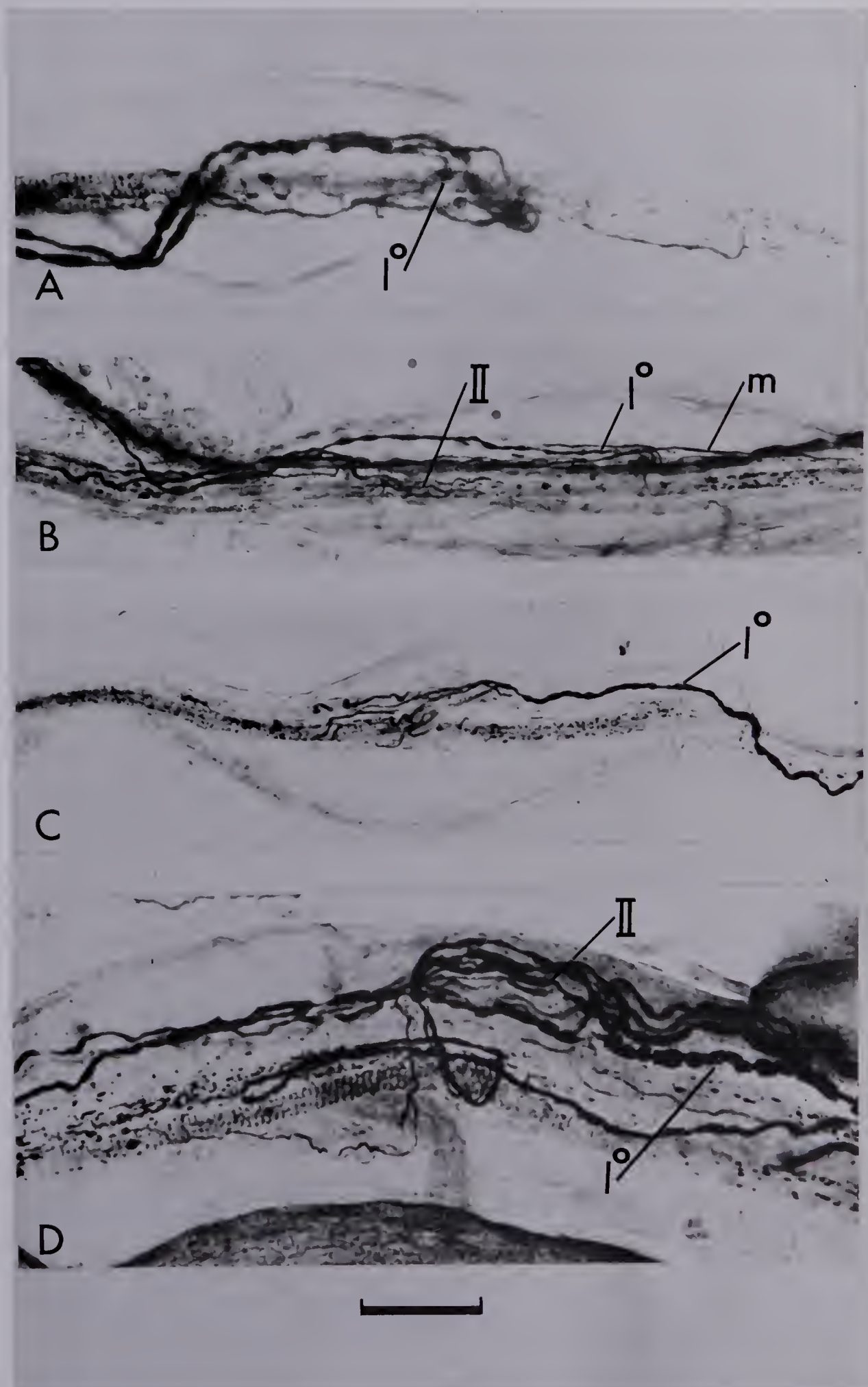


FIG. 27

Fig. 28. Primary sensory endings on nuclear bag and nuclear chain muscle fibers.

- A. The two types of muscle fibers, nuclear bag (nb) and nuclear chain (nc), can be identified by the shape of the nuclei (c.f. Fig. 10). Axons terminate as spiral endings on both types of fiber.
- B. Spiral primary endings can be seen at the right-hand side of the photograph.
- C. A spiral ending on a nuclear chain muscle fiber (nc).
- D. Spiral endings on a nuclear bag muscle fiber (nb).

Silver stain. Scale bar — A, C and D, 20 μ ; B, 40 μ .

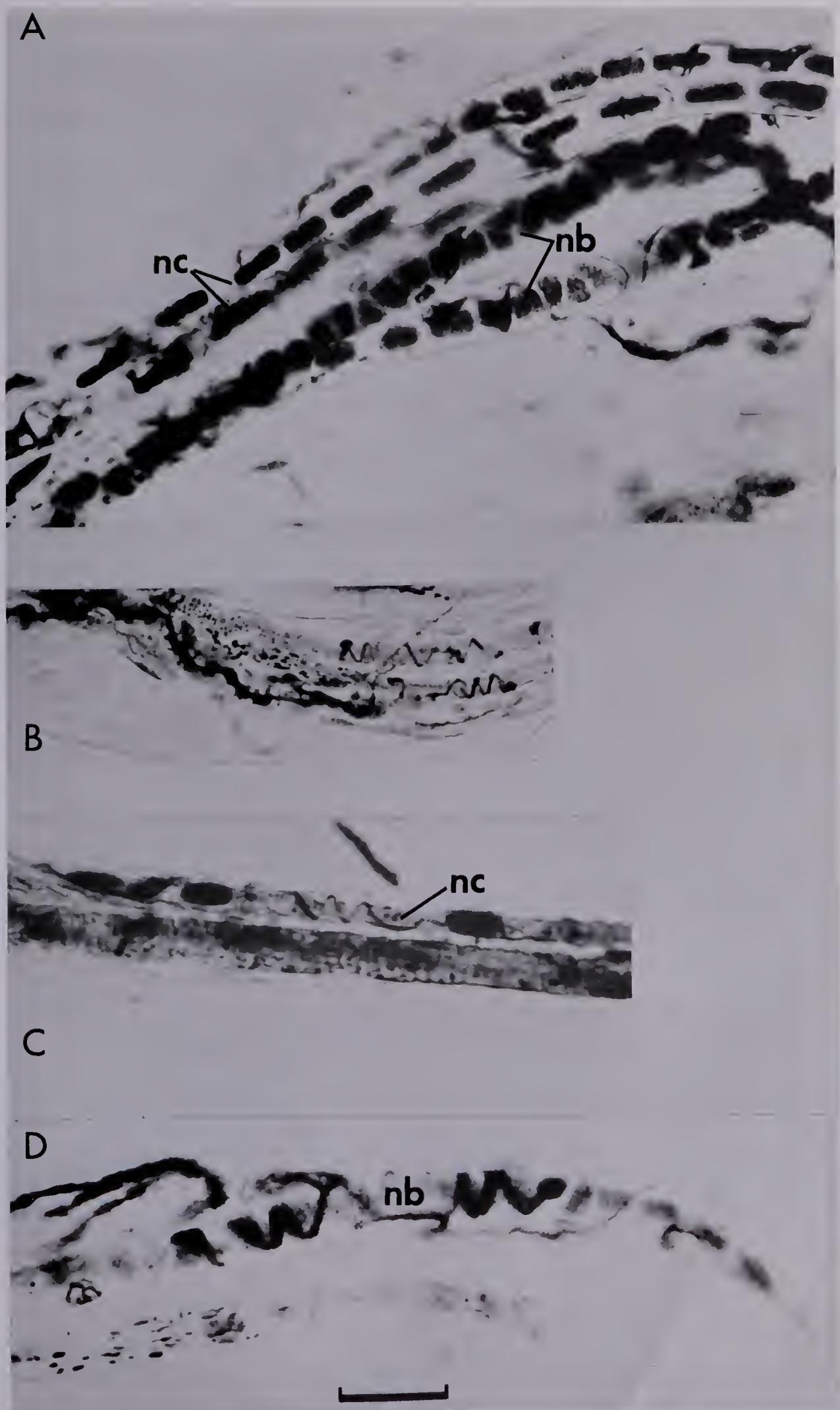


FIG. 28

Fig. 29. Low power photomicrographs to show an axon dividing to supply primary endings on two simple spindles.

- A. The sensory axon is indicated (I^0), it divides at the arrowhead. The upper branch of the axon innervates the equatorial region of the spindle shown, while the lower branch passes out of the field at the bottom of the picture.
- B. Both spindles are shown linked by their common sensory axon. The arrowhead indicates the point of division of the sensory nerve. A fusimotor axon entering the lower spindle is indicated (m).

Silver stain. Scale bar — A, 80 μ ; B, 200 μ .

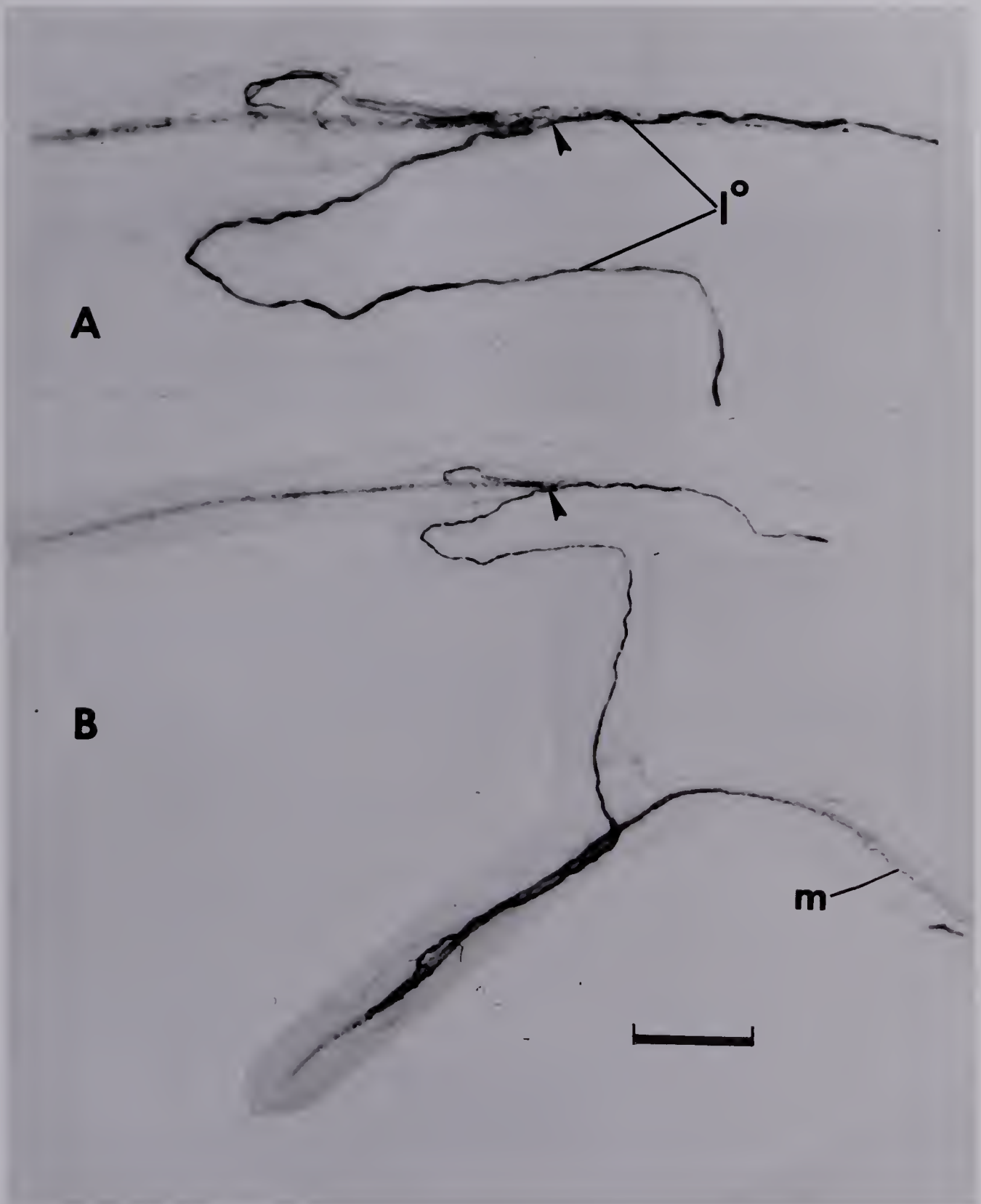


FIG. 29

Fig. 30. A. Capsular region of a spindle showing axons from primary (1°) and secondary (II) sensory endings as well as a fusimotor axon (m) traversing the capsule to enter the spindle pole to the right (not shown). The arrowheads outline the course of blood vessels, containing red blood cells, in the spindle capsule.

B. Capsular region of another spindle showing fine axons, as indicated by the arrowheads, ramifying throughout the capsular wall. These axons did not take part in the innervation of the intrafusal muscle fibers and are thought to be of autonomic origin.

C. Intact spindle showing sensory axons (S) from the intramuscular nerve trunk, entering the spindle at the capsular region. A fusimotor axon (m) is seen entering the spindle pole at the left.

A, B. Silver Stain.

C. Cole's gold chloride stain.

Scale bar — A and B, 20 μ ; C, 200 μ .

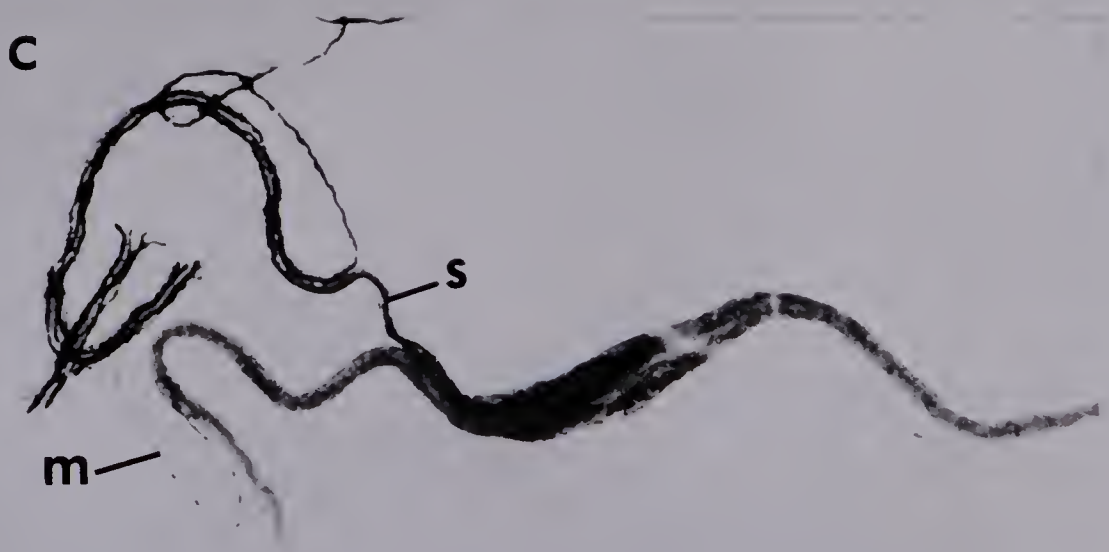
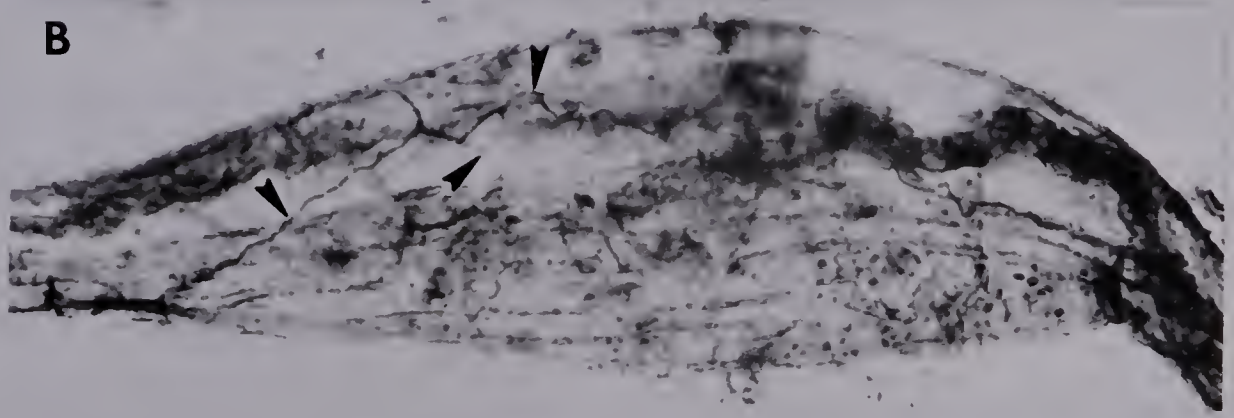
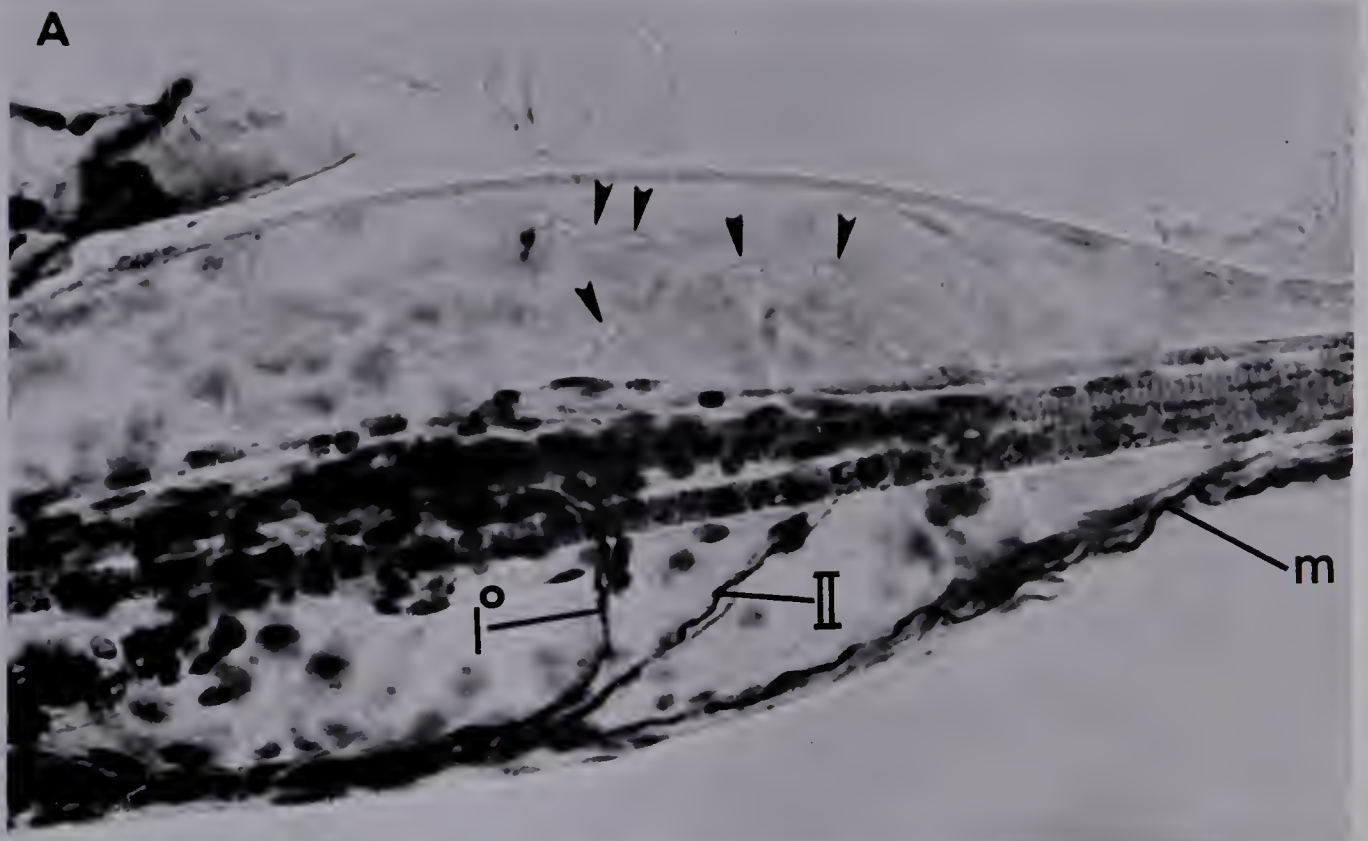
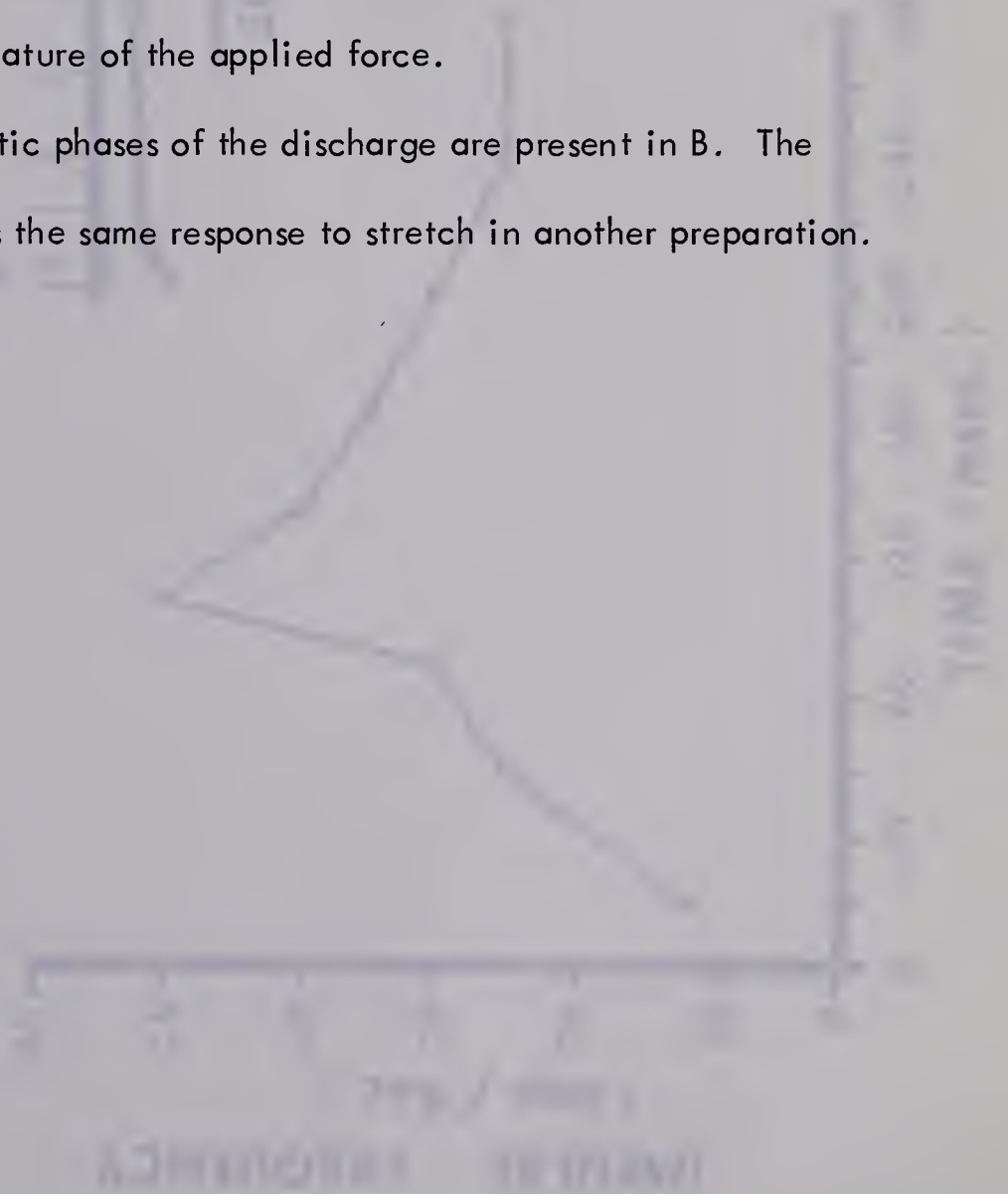


FIG. 30

Fig. 3I. Discharge characteristics of spindle afferents contained in plantar lumbrical muscle 5.

- A. Accelerated spindle discharge recorded from afferent nerve fibers in the lumbrical muscle nerve after application of Succinylcholine onto the muscle.
- B. Afferent discharge originating from a spindle contained in the lumbrical muscle and recorded from the muscle nerve. Muscle stretched 1mm from resting over 100 msec. Upper trace shows afferent response to stretch and lower trace the nature of the applied force.

Dynamic and static phases of the discharge are present in B. The impulse frequency plot shows the same response to stretch in another preparation. Temperature 32°C.



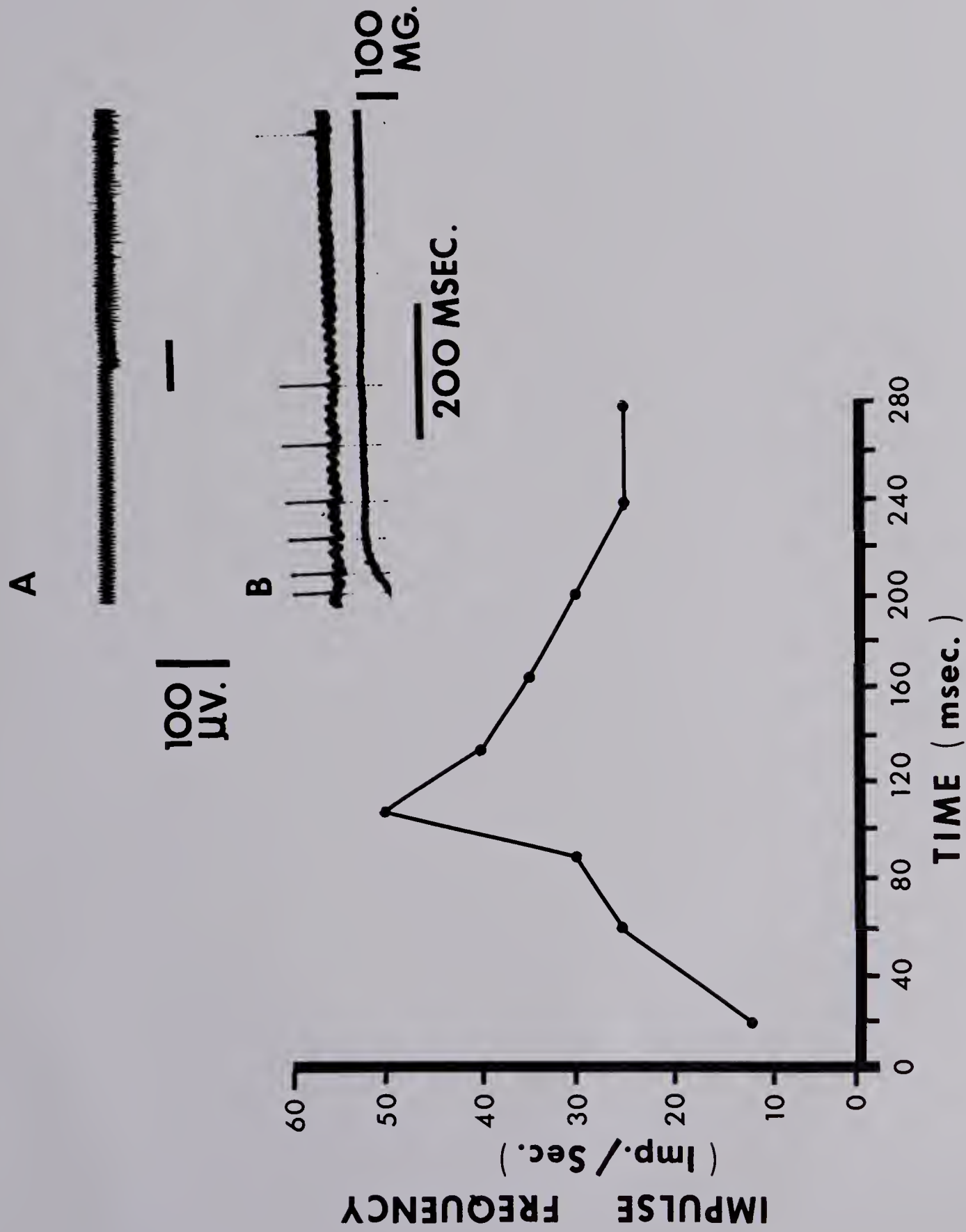


FIG. 31

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